(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
30 November 2000 (30.11.2000)

PCT

(10) International Publication Number
WO 00/71569 A1

(51) International Patent Classification[7]: C07K 1/02

(21) International Application Number: PCT/US00/14152

(22) International Filing Date: 23 May 2000 (23.05.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/322,762 26 May 1999 (26.05.1999) US
09/528,899 20 March 2000 (20.03.2000) US

(71) Applicant: ABBOTT LABORATORIES [US/US]; D377/Ap6D, 100 Abbott Park Road, Abbott Park, IL 60064-6050 (US).

(72) Inventors: TOLLE, John, C.; 2211 Sunnyside Drive, Kansasville, WI 53139 (US). CALIFANO, Jean-Christophe; 826 Washington Street, Apartment 2S, Evanston, IL 60202 (US). DHAON, Madhup, K.; 1337 Huntington Drive, Mundelein, IL 60060 (US). SACHS, Howard, A.; 1061 Belmar Lane, Buffalo Grove, IL 60089 (US). BLODGETT, James, K.; 410 Sommerset Drive, Grayslake, IL 60030 (US).

(74) Agents: SICKERT, Dugal, S. et al.; D377/AP6D, 100 Abbott Park Road, Abbott Park, IL 60064-6050 (US).

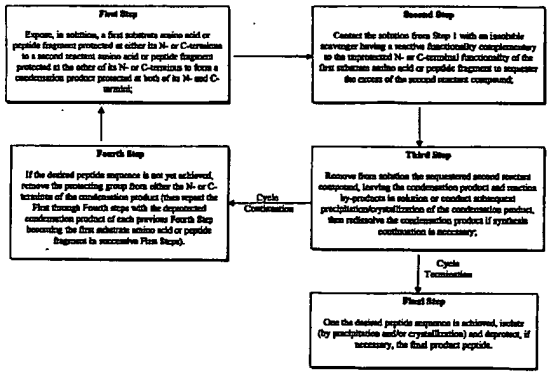
(81) Designated States *(national)*: CA, JP, MX.

(84) Designated States *(regional)*: European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published:
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: MINIMAL ISOLATION PEPTIDE SYNTHESIS PROCESS USING ION-EXCHANGE RESINS AS SCAVENGING AGENTS



(57) Abstract: A process for the production of a polypeptide having a pre-determined number and sequence of amino acid residues, comprising the steps of first exposing a first substrate amino acid or peptide fragment to a stoichiometric excess of a second reactant amino acid or peptide fragment to form a condensation product; second, contacting the reaction solution from the first step with an insoluble scavenger to sequester the excess of the second reactant amino acid or peptide fragment; third, removing from the solution the sequestered excess second reactant amino acid or peptide fragment; fourth, subjecting the reaction solution to a reaction which removes the protecting group from either the N- or C-terminus of the condensation product of the first step; and fifth, if necessary, repeating the first through fourth steps. The method is capable of large-scale production of peptides in solution, is not subject to the one-terminus-only limitation of the solid-phase method, possesses the "cleanliness" of the solid-phase method and, like the solid-phase method, is capable of automation. Most importantly, however, the method of the present invention does not require the frequent isolation of intermediates in a lengthy synthetic sequence nor, necessarily, the removal of all contaminating by-products from the reaction mixture prior to subsequent processing steps.

WO 00/71569 A1

MINIMAL ISOLATION PEPTIDE SYNTHESIS PROCESS USING ION-EXCHANGE RESINS AS SCAVENGING AGENTS

This application is a continuation-in-part of U.S. Patent Application Serial No. 09/322,762, filed May 26, 1999.

Technical Field

The present invention relates to synthetic chemical processes. More particularly, the present invention concerns a solution-phase process, particularly adapted to the production of commercial-scale quantities of polypeptides, which minimizes the requirement of isolation of intermediates.

Background of the Invention

Prior to the discovery by R. Bruce Merrifield in 1963 of a solid-phase method for synthesizing polypeptides (R. B. Merrifield, J. Am. Chem. Soc., 85: 2149-2154 (1963)), processes for the preparation of peptides containing more than a small number of amino acid residues were difficult and time-consuming. Since that time, the so-called Merrifield solid-phase technique has been used for the production of a large number of peptides.

The first stage of solid-phase peptide synthesis consists of the assembly of a peptide chain on a supporting insoluble polymer or resin by sequential reactions of protected amino acid derivatives. In a subsequent stage, the peptide chain is cleaved from the solid resin support with concurrent or subsequent cleavage of side-chain protecting groups to give the crude free peptide.

This technique has been adapted for use in two alternative methods. In the stationary solid-phase variant originally employed by Merrifield, the reagents and washing solvents are passed through a column of resin beads to which the growing peptide chain is attached and upon which it is supported. This synthetic method is limited to adding new amino acid residues to the growing peptide chain only at the N-terminus, since the chain is typically bound to the resin at its C-terminus. In a typical stationary solid-phase synthesis, individual amino acids are added to the N-terminus of the growing peptide chain until the desired polypeptide is obtained. In a less frequently used alternative, small peptide fragments consisting of multiple amino acid residues are added to the growing peptide bound to the resin.

In an alternative solid-phase peptide synthesis method, resin beads are slurried in a container and are exposed to successive reagent solutions or washing solvents with each being removed, typically through a filter in the bottom of the container, before the next is added. The slurry method of solid-phase peptide synthesis is likewise limited to one terminus-only synthesis of peptides. Both of the above-described variants of the solid-phase peptide synthesis method are

capable of being automated and are well suited to the preparation of milligram to multi-gram quantities of peptides. A number of automated solid-phase peptide synthesizers are commercially available which employ a microcomputer to open and close valves which control the sequence and duration of flow of various reagents and washing solvents delivered to a resin on which the growing peptide is supported. When quantities of peptides exceeding hundreds of grams are required, the solid-phase synthetic method is generally inadequate, and resort is made to the solution-phase method.

In the solution-phase method, polypeptides are pieced together by classical solution chemistry which facilitates joining together individual amino acids, or di-, tri-, tetra- or oligopeptide fragments of the final polypeptide in which sites of unwanted reaction have been appropriately protected. The smaller fragments are themselves similarly prepared by piecing together individual amino acids or smaller protected fragments, etc. By the judicious mapping of such a synthesis, it is possible to minimize the number of steps required for the production of a desired end-product. Unlike the solid-phase method, the solution-phase method is free of the one-terminus-only limitation on the synthesis of a peptide. In the solution-phase method, a fragment formed in a previous step by reaction of an unprotected N-terminal amino function can be reacted in a subsequent step at its unprotected C-terminal carboxyl function. This possibility is not open to the solid-phase method since, as stated above, the growing peptide is "blocked" at C-terminus by attachment to the supporting resin.

Solution-phase peptide synthesis, despite being free of the one-terminus-only synthesis limitation of solid-phase peptide synthesis, suffers from a shortcoming: the need to frequently isolate and purify the growing peptide. All reactions are carried out in solution, resulting in a mixture which contains the desired product as well as unwanted unreacted reagents and by-products. After several steps of the synthesis, the solution would become burdened with appreciable amounts of these contaminants which, if left in the solution, would affect subsequent steps of the synthesis or burden the isolation and purification of the desired end-product peptide. As a consequence, it is prudent and often necessary to isolate intermediate products as the peptide synthesis goes forward. Each such isolation adds to the cost and time of the synthesis and lowers the overall yield of the desired peptide.

Methods of preparing small amounts of large libraries of compounds have become available with the advent in recent years of combinatorial chemistry techniques. Recently, the use of resins to sequester excess reagents and by-products from combinatorial chemistry steps has been described by D. L. Flynn, *et al.*, J. Am. Chem. Soc., **119**: 4874-4881 (1997). In the method described there, the authors use so-called CMR/R, or complementary molecular

recognition/reactivity resins to purify products of each parallel step in a combinatorial chemistry scheme for preparing compound libraries. The process involves the synthesis of library members by solution-phase methods, followed by the removal of the solution-phase excess reactants, reagents, by-products and/or catalysts by incubation of the reaction mixture through CMR/R resins.

R. J. Booth, *et al.*, J. Am. Chem. Soc., **119**: 4882-4886 (1997) likewise describe the use of polystyrene-divinylbenzene-supported derivatives of *tris*-(2-aminoethyl)amine to quench excess reagents from crude reaction products obtained from solution-phase, parallel syntheses of amides in the construction of combinatorial libraries.

L. M. Gayo, *et al.*, Tetrahedron Letters, **38**(4): 513-516 (1997) describe the production of a combinatorial library of amides by first reacting, in parallel, a number of amines with a slight excess of an acid chloride to produce the desired amides. Following the reaction, the mixture is treated with water to convert the excess acid chloride to the corresponding carboxylic acid which, together with the HCl by-product of amide formation and acid chloride hydrolysis, is removed by a scavenging resin.

S. D. Brown, *et al.*, J. Am. Chem. Soc., **118**: 6331-6332 (1996) describe a "resin capture" technique for the combinatorial synthesis of tetra-substituted ethylene compounds using the reaction between *bis*(boryl)alkenes and alkyl halides. An intermediate product in the reaction is captured by a resin-bound aryl iodide to produce a desired product.

While these techniques describe creative ways of using functionalized resins to quench reactions or to capture excess reagents, by-products or desired products in combinatorial chemistry schemes, none has provided an effective method of preparing commercial-scale quantities of therapeutically useful compounds, particularly peptides. The particular problems of large-scale production, characteristic of commercial synthesis, are of little concern in the construction of a combinatorial library at the initial stages of drug discovery.

There is thus a need for improved methods of synthesizing commercial-scale quantities of peptides which makes the most of the advantages of both the solid-phase and the solution-phase methods while minimizing the disadvantages of each.

Summary of the Invention

In accordance with the present invention, an improved method of peptide synthesis is provided which capitalizes on the principal advantages of both the solid-phase and solution-phase methods of peptide synthesis. The method disclosed is capable of large-scale production of peptides in solution, is not subject to the one-terminus-only limitation of the solid-phase method.

possesses the "cleanliness" of the solid-phase method and, like the solid- phase method, is capable of automation. Most importantly, the method of the present invention does not require the frequent isolation of intermediates in a lengthy synthetic sequence nor, necessarily, the removal of all contaminants from the reaction mixture prior to subsequent processing steps.

In accordance with the principal embodiment of the present invention, there is provided a process for the synthesis of a polypeptide having a pre-determined number and sequence of amino acid residues. In its most general aspect, the process comprises sequentially the steps of first exposing, in solution, a first substrate amino acid or peptide fragment of the desired polypeptide product, the first substrate amino acid or peptide fragment being protected at either its N- or C-terminus, to a stoichiometric excess of a second reactant amino acid or peptide fragment of the desired polypeptide, the second reactant amino acid or peptide fragment being protected at the other of its N- or C-terminus, to form a condensation product of the substrate and reactant. The resulting condensation product is protected at both its N- and C-termini. In the second step of the process, the reaction solution is contacted with an insoluble scavenger having a reactive functionality complementary to the unprotected N- or C-terminal functionality of the first amino acid or peptide fragment, to sequester the excess of the second reactant amino acid or peptide fragment.

In the third step, the sequestered excess second reactant amino acid or peptide fragment is removed from the reaction solution, leaving the condensation product and reaction by-products in solution. Should it become necessary or desirable to decrease the volume of the reaction solution, which increases during the process of the present invention, the condensation product may be precipitated or crystallized, then place back in a solution of lesser volume. This solution, in the fourth step, is subjected to a reaction which removes the protecting group from either the N- or C-terminus of the condensation product of the first step. If, at this point, the desired polypeptide sequence is not yet achieved, the first through fourth steps are repeated as a cycle, with the deprotected condensation product of each previous fourth step becoming the substrate peptide fragment of each successive first step, until the desired peptide is produced. At the point at which the desired polypeptide sequence has been produced, the product is isolated and deprotected, if needed, of any terminal or side-chain protecting groups.

Description of the Drawing Figures

In the Drawing, which forms a part of the disclosure of the present invention:

FIGURE 1 is a schematic representation of the process steps for preparing polypeptides according to the method of the present invention.

FIGURE 2 is a schematic representation of a commercial-scale semi-automatic process apparatus for preparing polypeptides in accordance with the process of the present invention.

Detailed Description of the Invention

As used throughout this specification and the appended claims, the terms "protected" or "blocked," as it is applied to amino acids and peptides, have the meanings commonly accepted in the art. That is, a protected or blocked amino acid or peptide is one in which the reactive functionality of either or both the N-terminal amino group and/or the C-terminal carboxyl group have been blocked by reaction with a "blocking group" to prevent their reactivity. Additionally, other functional groups of the amino acid or peptide such as side- chain amino groups in lysine or hydroxy groups in serine, threonine, or tyrosine; and carboxyl groups in aspartic or glutamic acid residues may be blocked by appropriate blocking groups to prevent unwanted reactions.

Blocking groups suitable for the protection of amine, hydroxyl, and carboxyl functions are well known in the art. Blocking groups and methods for their attachment and cleavage are fully set out in "Protective Groups in Organic Synthesis," 2d Edition, by T. W. Greene, *et al.*, John Wiley & Sons, Inc., New York 1991.

In referring to various blocking groups, the acronym short-hand designations commonly employed by chemists is used throughout this specification. The definitions of the acronyms can be found, for example in T. W. Green, *et al., op. cit.* at pages xi-xvi. The acronym "OSu" refers to the moiety derived by removal of the hydroxyl hydrogen atom from N-hydroxysuccinimide. The blocking group known alternatively as "Cbz" or simply "Z" is the benzyloxycarbonyl protecting group. The blocking group known alternatively as "tBoc", or simply "Boc", is the tert-butyloxycarbonyl protecting group.

Referring to Figure 1, the general process of the present invention is depicted in schematic block diagram. In the first step of the process, the first amino acid or peptide fragment substrate, suitably protected except for either its N-terminal amino group or its C-terminal carboxyl function is exposed, in a suitable solvent, to the reactant amino acid or peptide fragment. The reactant may be a single amino acid or some peptide fragment of the end-product polypeptide, suitably protected at the other of its N-terminal amino group or C-terminal carboxyl group as well as at reactive side-chain functional groups. The carboxyl terminus of the N-terminal protected amino acid or peptide fragment is activated by conversion to an active ester of the types well known in the art. A preferred ester activating group for the process of the present invention is the N-hydroxysuccinimide ester group

(-OSu).

The protecting group for the N-terminus of the reactant or substrate amino acid or peptide fragment is a group which is "orthogonal" to protecting groups which are employed in protecting side-chain amino, hydroxyl, and carboxyl groups in either the reactant or substrate. Moreover, the N-terminal protecting group on the reactant or substrate amino acid or peptide fragment should be one which is easily removed under conditions which do not remove the side-chain protecting groups or the C-terminal blocking group of the other of the reactant or substrate. Two protecting groups are said to be "orthogonal" if chemical processes which are employed to remove one do not remove the other. A preferred N-terminal protecting group for the reactant or substrate amino acid or peptide fragment is one which is easily cleaved under conditions of hydrogenolysis, or catalytic hydrogenation. As sulfur is known to "poison" or inactivate hydrogenation catalysts, the preferred embodiment of the process of the present invention is limited to the synthesis of peptides which do not contain side-chain sulfhydryl or thioether groups; i.e., to the synthesis of non-cysteine-containing and non-methionine-containing peptides. The side-chain amino, hydroxyl and carboxyl blocking groups are selected from blocking groups well known in the art which are not cleaved under hydrogenolysis conditions. A preferred N-terminal amino protecting group for the reactant or substrate amino acids and peptide fragments utilized in the process of the present invention is the benzyloxycarbonyl group, sometimes termed the carbobenzoxy group, and denoted "Cbz" or simply "Z" in chemical shorthand. The Z group is easily cleaved by hydrogenolysis under mild conditions from the N-terminal amino groups of protected amino acids or peptides while leaving unaffected the less reactive protecting groups which have been used to protect side-chain functional groups. Preferred blocking groups for the C-terminus of the reactant or substrate amino acid or peptide fragment are simple ester groups such as the *tert*-butyl ester group and the like.

The substrate (unprotected at either its N-terminus or C-terminus) is allowed to react with the reactant (unprotected at the other of its N- or C-terminus) until analysis of aliquot samples periodically taken from the reaction mixture indicate substantially complete reaction. In the process of the present invention, the reactant is employed in stoichiometric excess to the amount of substrate in order to drive the condensation reaction to completion. Preferably, an amount greater than 1.0, up to about 1.1 moles of reactant amino acid or peptide fragment is employed per mole of substrate amino acid or peptide fragment. The amount of excess required in each particular coupling reaction will vary according to the chemical nature of the substrate and reactant to be coupled. However, it is within the skill of the process chemist to determine with a small-scale bench reaction the required molar ratio of reactant to substrate prior to committing to the cost of a large-scale preparation.

When the initial reaction between the substrate amino acid or peptide fragment and the reactant amino acid or peptide fragment is essentially complete, the reaction solution is contacted with a scavenger. The scavenger is both insoluble in the solvent system employed, and possesses a functionality which is complementary to that of the unprotected teminus of the substrate amino acid or peptide fragment. In the preferred embodiment of the process of the present invention, the substrate amino acid or peptide fragment is unprotected at its N-terminus and protected against reaction at its C-terminus. Correspondingly, the reactant amino acid or peptide fragment is protected at its N-terminus, preferably by a Z group, and is activated at its C-terminus. In this preferred embodiment of the process, the scavenger possesses an active amine functionality. The preferred scavenger is an amine-functionalized resin, such as aminomethyl-functionalized resins known in the art, particularly commercially available aminomethyl-modified styrene-divinylbenzene copolymers.

The method of contacting the reaction solution with the scavenger resin may be by either adding resin to the reaction vessel, or *vice versa*, with circulation of the reaction solution through a column of the resin being the preferred method. This method permits the repeated recirculation of the reaction solution through the resin column to ensure that the removal of excess reactant amino acid or peptide fragment is complete. Should it become necessary or desirable to decrease the reaction volume, which increases during each cycle of the process of the present invention, the condensation product may be precipitated or crystallized, then redissolved in a smaller volume of a suitable organic solvent.

In the next step of the process, the desired N-terminal or C-terminal blocking group of the condensation product of the first step of the process is removed. If, in a subsequent step, the reactant amino acid or peptide fragment is one in which the carboxyl terminus is unprotected, then the N-terminal protecting group of the condensation product is removed, and *vice versa*. In the preferred embodiment of the process of this invention, new segments, either single amino acids or short peptide fragments, are added to the growing polypeptide by utilizing a C-terminally activated reactant and an N-terminally unprotected substrate. This is because of the preferred N-terminal Z protecting group and the preferred C-terminal OSu activating group. Hence, in this step of the preferred embodiment of the process, the N-terminal Z group is removed from the condensation product.

The preferred method for this deprotection step is catalytic hydrogenolysis in the presence of a palladium catalyst at pressures ranging between atmospheric pressure and about 30 to 50 psi (206.8 kPa to 344.7 kPa). Preferred catalysts are those which can readily be removed from solution by filtration such as palladium supported on Deloxan® (organofunctional polysiloxane

polymers manufactured by DEGUSSA AG, Weissfrauenstrasse 9, Frankfort am Main, Germany), and palladium supported on carbon, alumina (Al_2O_3), or silica (SiO_2). The hydrogenolysis leaves in the solution, once the catalyst has been removed by filtration, only the N-hydroxysuccinimide by-product of the initial coupling reaction, the N-terminally deprotected condensation product, and toluene which, together with carbon dioxide is the by-product of the Z-group hydrogenolysis. (It should be noted that the N-hydroxysuccinimide and toluene by-products interfere with neither the catalytic hydrogenation deprotection step, nor subsequent condensations. The absence of any need for their removal in multi-step syntheses is a particular advantage of the preferred process of the present invention.)

If, following removal of excess reactant amino acid or peptide fragment, the desired end-product polypeptide sequence has been completely constructed, the process is over, except for isolation of the polypeptide and deprotection by conventional methods, if needed or desired. The isolation is further simplified in the preferred embodiment of the process of the present invention, since the only contaminants remaining in the reaction solution are N-hydroxysuccinimide, tertiary amine salt (if the substrate or reactant amino acid or peptide fragment is employed in salt form, requiring neutralization prior to coupling), and toluene, all of which are easily removed by simple precipitation or crystallization of the desired end-product polypeptide. Those skilled in the art will realize that, when a polypeptide bearing, for example, a free N-terminus is the desired end-product or when recrystallization alone can serve to remove excess reactant amino acid or peptide fragment, isolation may be carried our following completion of a particular fourth or first step, respectively.

If, however, as is the more general case, the desired end-product polypeptide synthesis in incomplete following removal of excess reactant amino acid or peptide fragment, the deprotection coupling and scavenging steps are repeated using the deprotected condensation product of each cycle as the substrate material of each subsequent cycle until the desired polypeptide sequence is achieved.

Referring to Figure 2, one embodiment of a semi-automatic commercial scale processing system is shown for use in carrying out the process of the present invention. The system **100** comprises a first reactor vessel **102** for carrying out amino acid/peptide fragment condensation or coupling reactions, a second reactor **106** for carrying out de-protecting reactions, a resin column **104**, a filter **108**, a product holding tank **110**, first and second solvent tanks, **112** and **114**, and a numeric processor control unit **116**. While stainless steel reaction vessels may be used, it is preferred that all reactor vessels and tanks be glass-lined. It should be noted that the process layout in Figure 2 is schematic only. Representation of the filter as a plate-and-frame filter press

and pumps as centrifugal pumps is merely for illustrative purposes. The selection of actual equipment to be employed would be within the skill and knowledge of a skilled chemical engineer.

The process described above would be carried out in an apparatus such as that shown in Figure 2 in a semi-automatic manner, essentially requiring only manual charging of reagents and solvents at various stages of the process. The operation of valves, pumps, and stirrers is controlled by the numeric processor control unit **116** which is programmed prior to each custom synthesis, based upon the materials employed. Table 1 below gives the process steps to aid in understanding the process flow through the apparatus. Control valves are opened or closed, as appropriate, for a given step of the process.

The first reaction step is carried out in reactor **102**, until essentially complete as indicated by analysis of samples from the reactor vessel. The samples may be physically withdrawn by the operator and analyzed or, in an alternative embodiment, analysis may be automated by means of a detector immersed in the reactor vessel content, sensitive to one or more of the reactant and substrate or a by-product of the coupling reaction. A signal from the sensor is sent to numeric processor **116** and used to determine the termination of the reaction step.

The mixture is stirred by means of stirrer **126** and the temperature is controlled to a level near ambient, generally less than about 30°C to about 35°C during the course of the reaction. The reactor vessel **102** may be of a type well known in the process arts for controlling chemical reaction temperature. For example, vessel **102** may be equipped with an immersion cooling coil, cooling coils welded to the outside of the vessel itself, or may be of a double-jacketed type which permits the circulation of a coolant between the walls of the vessel. In any of these embodiments, the circulation of coolant is controlled by a conventional feed-back loop connected to numeric controller **116** which reacts to a temperature sensor, not shown, immersed in the contents of reactor vessel **102** and uses that temperature to control the circulation of reactor vessel coolant.

When analysis of the contents of reactor **102** contents indicate substantially complete reaction, the contents are cycled through resin column **104** to remove excess reactant. By "cycled" is meant recirculating, by means of pump **120** and associated piping and valving, the contents of reactor vessel **102** through resin column **104**, and back into reactor vessel **102**. Excess reactant is taken up and held by chemical reaction with the resin, permitting only coupled product and reaction by-products to return to the reactor vessel.

The solution recycled through the column and reactor vessel is periodically analyzed for completeness of removal of excess reactant. As discussed above, this may be my means of physical removal of aliquot samples from the reactor vessel from time to time, or by a sensor immersed in the reactor vessel contents or fixed in-line in the piping connection between the

reactor vessel **102** and resin column **104**. The sensor is sensitive to excess reactant and is attached by feed-back control loop to numeric processor **116** and the signal from the recirculated product solution is used to control when the recirculation of the reaction solution through the resin column **104** may be stopped.

When analysis indicates that the reaction solution is substantially free of excess reactant, the numeric controller closes appropriate valves and opens others to transfer, by means of pump **122**, the eluate from resin column **102** to holding tank **114**. A first wash solvent from first solvent tank **112** is then circulated through resin column **104** and back to first solvent tank **112** to flush any remaining product from the column. This recirculating flush of column **104** may be for either a predetermined number of cycles or for a predetermined time, as controlled by numeric processor **116**. When the wash or flush of column **104** with the first solvent is complete, the first solvent wash solution, containing any product flushed from resin column **104**, is added to the contents of holding tank **114**.

Following the flush of resin column **104** by the first solvent, a second wash or flush of column **104** may be carried out if desired by employing a second solvent, initially held in second solvent tank **110**. As in the case of the wash of column **104** with the first solvent, the second solvent is recirculated between the column **104** and tank **110**, either for a pre-determined number of cycles, or for a pre-determined time, as controlled by numeric processor unit **116**. After complete flushing or washing of column **104**, the second solvent solution, containing any additional product washed from column **104** is added to the contents of holding tank **114**.

While the embodiment of the apparatus shown in Figure 2 depicts only two solvent wash tanks, **110** and **112**, it will be understood by those skilled in the chemical processing art that a third and subsequent tanks for additional solvents can be added as required. However, for most purposes, two tanks will suffice, with judicious choice of the wash solvents for each particular reaction step. Polar solvent can be employed as the wash solvent(s), with the limitation that the solvent must not be reactive with C-terminal activating groups, functional groups, or protecting groups on either the substrate or reactant. In addition, such solvents must serve to effectively solubilize both the substrate and the reactant. Suitable solvents include dimethylsulfoxide, dimethylformamide (DMF), N-methylpyrrolidone, and low molecular weight alcohols such as *iso*-propanol and the like, with cost and availability being concerns secondary to polarity and non-reactivity. Preferred solvents for the process of the present invention include dimethylformamide and *iso*-propanol.

At this step of the process, the holding tank **114** contains a solution comprising the coupled reaction product, together with the reaction solvent and the wash solvents, and (in the

preferred embodiment of the process) toluene, tertiary amine salt (if the substrate or reactant amino acid or peptide fragment is employed in a salt form requiring neutralization prior to coupling), and N-hydroxysuccinimide, but substantially free of contaminating excess reactant and substrate. The holding tank contents are next transferred by means of pump **124** to second reactor vessel **106** for removal of the Z-blocking group from the N-terminus of the coupled reaction product. As discussed above, the preferred benzyloxycarbonyl ("Z") protecting group is easily removed by hydrogenolysis under comparatively mild conditions. In Figure 2, second reactor **106** is shown as a hydrogenator fitted with gas inlet pipes **134**, **136**, and **138** and vent pipe **140**. Gas inlet pipes **134**, **136**, and **138** are connected to sources (not shown) of flush gases such as argon and nitrogen, and reaction gas, hydrogen. The flow of gases into and out of second reactor **106** is controlled by valves *1*, *2*, *3*, and *4* which, in turn are controlled by numeric processor **116**. The hydrogenator **116** is purged with flushing gases, initially flushed with hydrogen, and then charged at a higher pressure with hydrogen for the catalytic hydrogenolysis.

When the reaction is complete, appropriate valves are closed and opened to circulate the contents of the hydrogenator **106** through filter **108** to remove and collect the hydrogenation catalyst. From filter **108**, the reaction mixture is returned to first reactor vessel **102** for subsequent reaction steps, or to final product holding tank **114**.

Table 1

Process step 1:	Reactant and substrate charged to reactor vessel **102** and allowed to react.
Process step 2:	Reactor vessel **102** contents are recirculated through column **104**.
Process step 3:	Reactor vessel **102** contents are transferred to holding tank **114**.
Process step 4:	Cyclical first solvent flush of column **104** is carried out.
Process step 5:	First solvent flush is transferred to holding tank **114**.
Process step 6:	Cyclical second solvent flush of column **104** is carried out.
Process step 7:	Second solvent flush is transferred to holding tank **114**.
Process step 8:	Contents of holding tank **114** are transferred to second reactor vessel **106**.
Process step 9:	Second reactor vessel **106** is purged with first purge gas.
Process step 10:	Second reactor vessel **106** is purged with second purge gas.
Process step 11:	Second reactor vessel **106** is purged with hydrogen.
Process step 11:	Second reactor vessel **106** is pressurized with hydrogen and the hydrogenolysis reaction is allowed to proceed.
Process step 12:	Contents of second reactor vessel **106** are filtered to remove hydrogenation

catalyst and, if construction of the desired peptide is incomplete, transferred to first reactor vessel **102** for addition of next amino acid or peptide fragment to the growing peptide chain.

Process step 13: If construction of the desired peptide is incomplete, repeat steps 1-12 as required.

Process step 14: If construction of the peptide is complete, the contents of holding tank **114** from step 7 are collected for further processing.

The following examples are presented to enable one skilled in the art to better appreciate the process of the present invention. However, these examples are not to be read as limiting the scope of the invention as it is defined by the appended claims. Individual step numbers are designated in accordance with Figure 1. Numbering for cyclically repeated steps follows the format: step 1a, 2a, 3a and 4a for the first cycle of the process of the invention; step 1b, 2b, 3b and 4b for the second cycle; etc.

Example 1

Preparation of Benzyloxycarbonyl-Lysyl(*tert*-butyloxycarbonyl)-Alanyl-Phenylalanyl-Valyl-Lysyl(*tert*-butyloxycarbonyl)-Isoleucyl-Leucyl-Lysyl(*tert*-butyloxycarbonyl)-Lysine(*tert*-butyloxycarbonyl)-methyl Ester

Step 1a) Preparation of Benzyloxycarbonyl-Lysyl(*tert*-butyloxycarbonyl)-Lysine(*tert*-butyloxycarbonyl)-methyl Ester

Lys(Boc)-OMe.HCl (2.96 g, 10 mmol), and Z-Lys(Boc)-OSu (5.25 g, 11 mmol) are mixed with 40 mL (41.32 g) of *N*-Methyl-2-pyrrolidinone (NMP) in a 50 mL glass reactor, and the mixture is stirred at room temperature until the solids are dissolved. Diisopropylethylamine (DIEA) (1.24 g, 10.50 mmol) is added slowly to the reactor contents over a period of from about fifteen to thirty minutes. The resulting mixture is allowed to react at room temperature for about two hours. After this period of time, an aliquot sample of the reaction mixture is taken for analysis of the completeness of the reaction. If the analysis indicates that the reaction is incomplete, the reaction mixture is stirred at room temperature for an additional hour and the reaction mixture is analyzed again. When this analysis indicates the coupling reaction is complete, the reactor contents are held at room temperature with slow stirring.

Steps 2a-3a) Scavenging/Sequestration of Excess Reactant Z-Lys(Boc)-OSu

Aminomethyl resin (2 g) is mixed with 30 mL (30.99 g) of NMP in a second reactor vessel. The resulting mixture is stirred at room temperature until a homogeneous slurry is obtained. The resin/NMP slurry is charged to a glass column, the resin slurry is allowed to settle into a packed bed, and any excess NMP is drained from the column.

The NMP reaction mixture containing the Z-Lys(Boc)-Lys(Boc)-OMe product and excess Z-Lys(Boc)-OSu reagent from Step 1a is then circulated repetitively through the resin column for about one hour in order to remove excess Z-Lys(Boc)-OSu. Circulation is continued, if necessary, until analysis indicates complete removal of the excess reactant. At this point, the NMP reaction solution containing the blocked Z-Lys(Boc)-Lys(Boc)-OMe dipeptide product is set aside. The resin column is then washed by recirculating 15 mL (11.75 g) of *iso*-propanol through the column for about thirty minutes. The *iso*-propanol solution, containing blocked dipeptide product washed from the column, is set aside. The column is next washed by recirculating 30 mL (30.99 g) of NMP through the column for about thirty minutes. The NMP solution, containing blocked dipeptide product washed from the column, is set aside. The resin column is given a final wash by recirculating 15 mL (11.75 g) of *iso*-propanol through the column for about thirty minutes. The NMP reaction solution of blocked dipeptide, the NMP wash solution, and the two *iso*-propanol wash solutions are combined.

Step 4a) N-Terminal Deprotection of the Benzyloxycarbonyl-Lysyl(*tert*-butyloxycarbonyl)-Lysine(*tert*-butyloxycarbonyl)-methyl Ester Ester Product of Step 1a

Palladium-Deloxan® (0.60 g) and *para*-toluene sulfonic acid (pTSA) (1.90 g, 10 mmol) are placed in a 250 mL hydrogenator vessel. The hydrogenator vessel is flooded with argon, and the combined NMP/*iso*-propanol solutions from Steps 2a-3a are charged to the hydrogenator vessel. The vessel is sealed and evacuated to a pressure of 20-25 inches of mercury (67.7 - 84.6 kPa) and purged three times with hydrogen. Hydrogen is then charged to the hydrogenator vessel to a pressure of 35-45 psi (234.5-310.3 kPa) and the mixture is stirred at about 30°C for about 2 hours.

The hydrogen is then vented from the hydrogenator vessel, and the vessel is purged twice with nitrogen. An aliquot sample of the reaction mixture is taken for analysis of the completeness of reaction. If the analysis indicates incomplete reaction, the hydrogenator vessel is purged twice with hydrogen, recharged with hydrogen to a pressure of 35-45 psi (234.5-310.3 kPa), and the mixture is again stirred at a temperature of about 30°C for an additional hour. The hydrogenator vessel is evacuated and then purged twice with nitrogen, and an aliquot sample of the reaction mixture is again taken for analysis of the completeness of reaction. The above steps of

hydrogenation and reaction mixture analysis are repeated until the analysis indicates substantial completion of the hydrogenolysis reaction.

Upon completion of the hydrogenolysis reaction, the hydrogen is vented from the hydrogenator vessel, the vessel is purged with nitrogen, and the vessel contents are filtered to remove the catalyst. The hydrogenator vessel is rinsed with NMP, and the rinse solution is added to the reaction mixture filtrate.

Step 1b) Preparation of Benzyloxycarbonyl-Leucyl-Lysyl(*tert*-butyloxycarbonyl)-Lysine(*tert*-butyloxycarbonyl)-methyl Ester

Z-Leu-OSu (3.98 g, 11 mmol), DIEA (1.29 g, 11 mmol) and the NMP/*iso*-propanol solution of the N-terminal deprotected dipeptide product of Step 4a (10 mmol, assuming complete reaction) are placed in a 1-liter glass reactor, and the mixture is stirred at room temperature until all solids are dissolved. The mixture is then stirred and allowed to react at room temperature for about two hours. At the end of this time, an aliquot sample of the reaction mixture is taken for analysis of the completeness of reaction. If the analysis indicates that the reaction is incomplete, the reaction mixture is stirred at room temperature for an additional hour and the reaction mixture is analyzed again. When the analysis indicates the coupling reaction is complete, the reactor contents are held at room temperature with slow stirring.

Steps 2b-3b) Scavenging/Sequestration of Excess Reactant Z-Leu-OSu

A column of aminomethyl resin (2 g) is prepared as described above in Step 2a-3a, and the NMP/*iso*-propanol reaction mixture of the blocked tripeptide from Step 1b is circulated repetitively through the resin column for about one hour, periodically analyzing the column eluate for absence of excess Z-Leu-OSu. When the analysis indicates substantially pure product, the NMP/*iso*-propanol reaction solution of the blocked tripeptide is set aside, and the column is washed by recirculating 15 mL (11.75 g) of *iso*-propanol through the column for about thirty minutes. The *iso*-propanol solution, containing blocked tripeptide product washed from the column, is set aside. The column is next washed by recirculating 30 mL (30.99 g) of NMP through the column for about thirty minutes. The NMP solution, containing blocked tripeptide product washed from the column, is set aside. The NMP/*iso*-propanol reaction solution of blocked tripeptide product and the *iso*-propanol and NMP wash solutions are combined.

Step 4b) N-Terminal Deprotection of the Benzyloxycarbonyl-Leucyl-Lysyl(*tert*-butyloxycarbonyl)-Lysine(*tert*-butyloxycarbonyl)-methyl Ester Product of Step 1b

Using the same process as that of Step 4a, but without the addition of pTSA, the benzyloxycarbonyl protecting group on the Z-Leu-Lys(Boc)-Lys(Boc)-OMe tripeptide product of Step 1b is removed by hydrogenolysis.

Step 1c) Preparation of Benzyloxycarbonyl-Isoleucyl-Leucyl-Lysyl(*tert*-butyloxycarbonyl)-Lysine(*tert*-butyloxycarbonyl)-methyl Ester

Z-Ile-OSu (3.98 g, 11 mmol) is mixed in a 1-liter glass reactor vessel with the NMP/*iso*-propanol solution of the N-terminal deprotected tripeptide product from Step 4b. The mixture is stirred at room temperature until all solids are dissolved. The resulting mixture is allowed to react at room temperature for at least 2 hours, at which time an aliquot sample of the reaction mixture is analyzed for completeness of reaction. If the analysis indicates that the reaction is incomplete, the reaction mixture is stirred at room temperature for an additional hour and the reaction mixture is analyzed again. When analysis indicates the coupling reaction is complete, the reactor contents are held at room temperature with slow stirring.

Steps 2c-3c) Scavenging/Sequestration of Excess Reactant Z-Ile-OSu

A column of aminomethyl resin (2 g) is prepared as described above in Step 2a-3a, and the NMP/*iso*-propanol reaction mixture of the blocked tetrapeptide from Step 1c is then circulated repetitively through the resin column for about one hour in order to remove excess Z-Ile-OSu. Circulation is continued, if necessary, until analysis indicates complete removal of the excess reactant. At this point, the NMP/*iso*-propanol reaction solution containing the blocked tetrapeptide product is set aside. The resin column is then washed by recirculating 15 mL (11.75 g) of *iso*-propanol through the column for about thirty minutes. The *iso*-propanol solution, containing blocked tetrapeptide product washed from the column, is set aside. The column is next washed by recirculating 30 mL (30.99 g) of NMP through the column for about thirty minutes. The NMP solution, containing blocked tetrapeptide product washed from the column, is set aside. The resin column is given a final wash by recirculating 15 mL (11.75 g) of *iso*-propanol through the column for about thirty minutes. The NMP/*iso*-propanol reaction solution of blocked tetrapeptide product, the NMP wash solution, and the two *iso*-propanol wash solutions are combined.

Step 4c) N-Terminal Deprotection of the Benzyloxycarbonyl-Isoleucyl-Leucyl-Lysyl(*tert*-butyloxycarbonyl)-Lysine(*tert*-butyloxycarbonyl)-methyl Ester Product of Step 1c

Using the same process as that of Step 4a, but without the addition of pTSA, the benzyloxycarbonyl protecting group on the Z-Ile-Leu-Lys(Boc)-Lys(Boc)-OMe tetrapeptide product of Step 1c is removed by hydrogenolysis.

Step 1d) Preparation of Benzyloxycarbonyl-Lysine(*tert*-butyloxycarbonyl)-Isoleucyl-Leucyl-Lysyl(*tert*-butyloxycarbonyl)-Lysine(*tert*-butyloxycarbonyl)-methyl Ester

Z-Lys(Boc)-OSu (5.25 g, 11 mmol) is mixed in a 1-liter glass reactor vessel with the NMP/*iso*-propanol solution of the N-terminal deprotected tetrapeptide product from Step 4c. The mixture is stirred at room temperature until all solids are dissolved. The resulting mixture is allowed to react at room temperature for at least 2 hours, at which time an aliquot sample of the reaction mixture is analyzed for completeness of reaction. If the analysis indicates that the reaction is incomplete, the reaction mixture is stirred at room temperature for an additional hour and the reaction mixture is analyzed again. When analysis indicates the coupling reaction is complete, the reactor contents are held at room temperature with slow stirring.

Steps 2d-3d) Scavenging/Sequestration of Excess Reactant Z-Lys(Boc)-OSu and Precipitation/Crystallization of Condensation Product

A column of aminomethyl resin (2 gm) is prepared as described above in Step 2a-3a, and the NMP/*iso*-propanol reaction mixture of the blocked pentapeptide from Step 1d is then circulated repetitively through the resin column for about one hour in order to remove excess Z-Lys(Boc)-OSu. Circulation is continued, if necessary, until analysis indicates complete removal of the excess reactant. At this point, the NMP/*iso*-propanol reaction solution containing the blocked pentapeptide product is set aside. The resin column is then washed by recirculating 15 mL (11.75 g) of *iso*-propanol through the column for about thirty minutes. The *iso*-propanol solution, containing blocked pentapeptide product washed from the column, is set aside. The column is next washed by recirculating 30 mL (30.99 g) of NMP through the column for about thirty minutes. The NMP solution, containing blocked pentapeptide product washed from the column, is set aside. The resin column is given a final wash by recirculating 15 mL (11.75 g) of *iso*-propanol through the column for about thirty minutes. The NMP/*iso*-propanol reaction solution of blocked pentapeptide product, the NMP wash solution, and the two *iso*-propanol wash solutions are combined.

To the NMP/*iso*-propanol (± 350 mL) solution of the blocked pentapeptide is added precooled water (150 mL), causing precipitation/crystallization of the Z-Lys(Boc)-Ile-Leu-Lys(Boc)-Lys(Boc)-OMe pentapeptide product. The mixture is stirred for 24 hours, after which

time the pentapeptide is isolated by filtration. The resulting solid is reslurried with water (200mL) for 4 hours and filtered again, then dried under vacuum. The dried solid is recrystallized from MeOH/EtOAc (40/45 mL) at 70 °C, then stirred for 2 hours at room temperature in the mother liquor. The recrystallized solid is isolated by filtration, rinsed twice with a volume of EtOAc (15 mL) and dried under vacuum. The yield of the Z-Lys(Boc)-Ile-Leu-Lys(Boc)-Lys(Boc)-OMe is 8.80 g (74% of theoretical yield). The MeOH/EtOAc filtrate is concentrated to dryness with concomitant solidification of additional Z-Lys(Boc)-Ile-Leu-Lys(Boc)-Lys(Boc)-OMe. The resulting solid is recrystallized in MeOH/EtOAc (10/10 mL), filtered and rinsed with two volumes of EtOAc (8 mL), then dried under vacuum. The solid obtained (0.48 g) is combined with the Z-Lys(Boc)-Ile-Leu-Lys(Boc)-Lys(Boc)-OMe (8.80 g) from the first recrystallization to afford a total of 9.28 g (78% of theoetical yield).

Step 4d) N-Terminal Deprotection of the Benzyloxycarbonyl-Lysyl(*tert*-butyloxycarbonyl)-Isoleucyl-Leucyl-Lysyl(*tert*-butyloxycarbonyl)-Lysine(*tert*-butyloxycarbonyl)-methyl Ester Product of Step 3d

Z-Lys(Boc)-Ile-Leu-Lys(Boc)-Lys(Boc)-OMe (9.28 g, 0.86 mmol) is mixed with 60 mL of NMP in a 1-liter glass reactor. Then, using the same process as that of Step 4a, but without the addition of pTSA, the benzyloxycarbonyl protecting group on the N-terminus of the pentapeptide product (of Step 3d) is removed by hydrogenolysis.

Step 1e) Preparation of Benzyloxycarbonyl-Valyl-Lysine(*tert*-butyloxycarbonyl)-Isoleucyl-Leucyl-Lysyl(*tert*-butyloxycarbonyl)-Lysine(*tert*-butyloxycarbonyl)-methyl Ester

Z-Val-OSu (3.30 g, 9.50 mmol) is mixed in a 1-liter glass reactor vessel with the NMP solution of the N-terminal deprotected pentapeptide product from Step 4d. The mixture is stirred at room temperature until all solids are dissolved. The resulting mixture is allowed to react at room temperature for at least 2 hours, at which time an aliquot sample of the reaction mixture is analyzed for completeness of reaction. If the analysis indicates that the reaction is incomplete, the reaction mixture is stirred at room temperature for an additional hour and the reaction mixture is analyzed again. When analysis indicates the coupling reaction is complete, the reactor contents are held at room temperature with slow stirring.

Steps 2e-3e) Scavenging/Sequestration of Excess Reactant Z-Val-OSu

A column of aminomethyl resin (1.60 g) is prepared as described above in Step 2a-3a, and the NMP reaction mixture of the blocked hexapeptide from Step 1e is then circulated repetitively through the resin column for about one hour in order to remove excess Z-Val-OSu. Circulation is

continued, if necessary, until analysis indicates complete removal of the excess reactant. At this point, the NMP reaction solution containing the blocked hexapeptide product is set aside. The resin column is then washed by recirculating 15 mL (11.75 g) of *iso*-propanol through the column for about thirty minutes. The *iso*-propanol solution, containing blocked hexapeptide product washed from the column, is set aside. The column is next washed by recirculating 30 mL (30.99 g) of NMP through the column for about thirty minutes. The NMP solution, containing blocked hexapeptide product washed from the column, is set aside. The resin column is given a final wash by recirculating 15 mL (11.75 g) of *iso*-propanol through the column for about thirty minutes. The NMP reaction solution of blocked hexapeptide, the NMP wash solution, and the two *iso*-propanol wash solutions are combined.

Step 4e) N-Terminal Deprotection of the Benzyloxycarbonyl-Valyl-Lysyl(*tert*-butyloxycarbonyl)-Isoleucyl-Leucyl-Lysyl(*tert*-butyloxycarbonyl)-Lysine(*tert*-butyloxycarbonyl)-methyl Ester Product of Step 1e

Using the same process as that of Step 4a, but without the addition of pTSA, the benzyloxycarbonyl protecting group on the Z-Val-Lys(Boc)-Ile-Leu-Lys(Boc)-Lys(Boc)-OMe hexapeptide product of Step 1e is removed by hydrogenolysis.

Step 1f) Preparation of Benzyloxycarbonyl-Phenylalanyl-Valyl-Lysine(*tert*-butyloxycarbonyl)-Isoleucyl-Leucyl-Lysyl(*tert*-butyloxycarbonyl)-Lysine(*tert*-butyloxycarbonyl)-methyl Ester

Z-Phe-OSu (3.77 g, 9.50 mmol) is mixed in a 1-liter glass reactor vessel with the NMP/*iso*-propanol solution of the N-terminal deprotected hexapeptide product from Step 4e. The mixture is stirred at room temperature until all solids are dissolved. The resulting mixture is allowed to react at room temperature for at least 2 hours, at which time an aliquot sample of the reaction mixture is analyzed for completeness of reaction. If the analysis indicates that the reaction is incomplete, the reaction mixture is stirred at room temperature for an additional hour and the reaction mixture is analyzed again. When analysis indicates the coupling reaction is complete, the reactor contents are held at room temperature with slow stirring.

Steps 2f-3f) Scavenging/Sequestration of Excess Reactant Z-Phe-OSu

A column of aminomethyl resin (1.60 g) is prepared as described above in Step 2a-3a, and the NMP/iso-propanol reaction mixture of the blocked heptapeptide from Step 1f is then circulated repetitively through the resin column for about one hour in order to remove excess Z-Phe-OSu. Circulation is continued, if necessary, until analysis indicates complete removal of the excess reactant. At this point, the NMP/*iso*-propanol reaction solution containing the blocked

heptapeptide product is set aside. The resin column is then washed by recirculating 15 mL (11.75 g) of *iso*-propanol through the column for about thirty minutes. The *iso*-propanol solution, containing blocked heptapeptide product washed from the column, is set aside. The column is next washed by recirculating 30 mL (30.99 g) of NMP through the column for about thirty minutes. The NMP solution, containing blocked heptapeptide product washed from the column, is set aside. The resin column is given a final wash by recirculating 15 mL (11.75 g) of *iso*-propanol through the column for about thirty minutes. The NMP/*iso*-propanol reaction solution of blocked heptapeptide, the NMP wash solution, and the two *iso*-propanol wash solutions are combined.

Step 4f)N-Terminal Deprotection of the Benzyloxycarbonyl-Phenylalanyl-Valyl-Lysyl(*tert*-butyloxycarbonyl)-Isoleucyl-Leucyl-Lysyl(*tert*-butyloxycarbonyl)-Lysine(*tert*-butyloxycarbonyl)-methyl Ester Product of Step 1f

Using the same process as that of Step 4a, but without the addition of pTSA, the benzyloxycarbonyl protecting group on the Z-Phe-Val-Lys(Boc)-Ile-Leu-Lys(Boc)-Lys(Boc)-OMe heptapeptide product of Step 1f is removed by hydrogenolysis.

Step 1g) Preparation of Benzyloxycarbonyl-Alanyl-Phenylalanyl-Valyl-Lysine(*tert*-butyloxycarbonyl)-Isoleucyl-Leucyl-Lysyl(*tert*-butyloxycarbonyl)-Lysine(*tert*-butyloxycarbonyl)-methyl Ester

Z-Ala-OSu (3.04 g, 9.50 mmol) is mixed in a 1-liter glass reactor vessel with the NMP/*iso*-propanol solution of the N-terminal deprotected heptapeptide product from Step 4f. The mixture is stirred at room temperature until all solids are dissolved. The resulting mixture is allowed to react at room temperature for at least 2 hours, at which time an aliquot sample of the reaction mixture is analyzed for completeness of reaction. If the analysis indicates that the reaction is incomplete, the reaction mixture is stirred at room temperature for an additional hour and the reaction mixture is analyzed again. When this analysis indicates the coupling reaction is complete, the reactor contents are held at room temperature with slow stirring.

Steps 2g-3g) Scavenging/Sequestration of Excess Reactant Z-Ala-OSu

A column of aminomethyl resin (1.60 g) is prepared as described above in Step 2a-3a, and the NMP/*iso*-propanol reaction mixture of the blocked octapeptide from Step 1g is then circulated repetitively through the resin column for about one hour in order to remove excess Z-Ala-OSu. Circulation is continued, if necessary, until analysis indicates complete removal of the excess reactant. At this point, the NMP/*iso*-propanol reaction solution containing the blocked octapeptide product is set aside. The resin column is then washed by recirculating 15 mL (11.75 g) of *iso*-

propanol through the column for about thirty minutes. The *iso*-propanol solution, containing blocked octapeptide product washed from the column, is set aside. The column is next washed by recirculating 30 mL (30.99 g) of NMP through the column for about thirty minutes. The NMP solution, containing blocked octapeptide product washed from the column, is set aside. The resin column is given a final wash by recirculating 15 mL (11.75 g) of *iso*-propanol through the column for about thirty minutes. The NMP/*iso*-propanol reaction solution of blocked octapeptide, the NMP wash solution, and the two *iso*-propanol wash solutions are combined.

Step 4g N-Terminal Deprotection of the Benzyloxycarbonyl-Alanyl-Phenylalanyl-Valyl-Lysyl(*tert*-butyloxycarbonyl)-Isoleucyl-Leucyl-Lysyl(*tert*-butyloxycarbonyl)-Lysine(*tert*-butyloxycarbonyl)-methyl Ester Product of Step 1g

Using the same process as that of Step 4a, but without the addition of pTSA, the benzyloxycarbonyl protecting group on the Z-Ala-Phe-Val-Lys(Boc)-Ile-Leu-Lys(Boc)-Lys(Boc)-OMe octapeptide product of Step 1g is removed by hydrogenolysis.

Step 1h) Preparation of Benzyloxycarbonyl-Lysyl(*tert*-butyloxycarbonyl-Alanyl-Phenylalanyl-Valyl-Lysine(*tert*-butyloxycarbonyl)-Isoleucyl-Leucyl-Lysyl(*tert*-butyloxycarbonyl)-Lysine(*tert*-butyloxycarbonyl)-methyl Ester

Z-Lys(Boc)-OSu (4.54 g, 9.50 mmol) is mixed in a 1-liter glass reactor vessel with the NMP/*iso*-propanol solution of the N-terminal deprotected octapeptide product from Step 4g. The mixture is stirred at room temperature until all solids are dissolved. The resulting mixture is allowed to react at room temperature for at least 2 hours, at which time an aliquot sample of the reaction mixture is analyzed for completeness of reaction. If the analysis indicates that the reaction is incomplete, the reaction mixture is stirred at room temperature for an additional hour and the reaction mixture is analyzed again. When this analysis indicates the coupling reaction is complete, the reactor contents are held at room temperature with slow stirring.

Steps 2h-3h) Scavenging/Sequestration of Excess Reactant Z-Lys(Boc)-OSu

A column of aminomethyl resin (1.60 g) is prepared as described above in Step 2a-3a, and the NMP/*iso*-propanol reaction mixture of the blocked nonapeptide from Step 1h is then circulated repetitively through the resin column for about one hour in order to remove excess Z-Lys(Boc)-OSu. Circulation is continued, if necessary, until analysis indicates complete removal of the excess reactant. At this point, the NMP/*iso*-propanol reaction solution containing the blocked nonapeptide product is set aside. The resin column is then washed by recirculating 15 mL (11.75 g) of *iso*-propanol through the column for about thirty minutes. The *iso*-propanol solution,

containing blocked nonapeptide product washed from the column, is set aside. The column is next washed by recirculating 30 mL (30.99 g) of NMP through the column for about thirty minutes. The NMP solution, containing blocked nonapeptide product washed from the column, is set aside. The resin column is given a final wash by recirculating 15 mL (11.75 g) of *iso*-propanol through the column for about thirty minutes. The NMP/*iso*-propanol reaction solution of blocked nonapeptide, the NMP wash solution, and the two *iso*-propanol wash solutions are combined.

Step 5) Isolation of Benzyloxycarbonyl-Lysyl(*tert*-butyloxycarbonyl-Alanyl-Phenylalanyl-Valyl-Lysine(*tert*-butyloxycarbonyl)-Isoleucyl-Leucyl-Lysyl(*tert*-butyloxycarbonyl)-Lysine(*tert*-butyloxycarbonyl)-methyl Ester

To the NMP/*iso*-propanol solution of the blocked nonapeptide from Step 2h-3h is added precooled water (300 mL), causing precipitation/crystallization of the Z-Lys(Boc)-Ala-Phe-Val-Lys(Boc)-Ile-Leu-Lys(Boc)-Lys(Boc)-OMe nonapeptide product. The mixture is stirred for 24 hours, after which time the nonapeptide is isolated by filtration. The resulting solid is reslurried with water (250mL) for 4 hours and filtered again, then dried under vacuum. The yield of the Z-Lys(Boc)-Ala-Phe-Val-Lys(Boc)-Ile-Leu-Lys(Boc)-Lys(Boc)-OMe is 13.28 g (94% of theoretical yield from Z-Lys(Boc)-Ile-Leu-Lys(Boc)-Lys(Boc)-OMe, 82% overall yield).

Example 2

Preparation of Benzyloxycarbonyl-Lysyl(*tert*-butyloxycarbonyl)-Phenylalanyl-Leucyl-Lysyl(*tert*-butyloxycarbonyl)-Lysyl(*tert*-butyloxycarbonyl)-Alanyl-Lysyl(*tert*-butyloxycarbonyl)-Lysyl(*tert*-butyloxycarbonyl)-Phenylalanyl-Glycine-methyl Ester

Step 1a) Preparation of Benzyloxycarbonyl-Phenylalanyl-Glycine-methyl Ester

Gly-OMe.HCl (6.25 g, 50 mmol) and Z-Phe-OSu (21.80 g, 55 mmol) are mixed with 60 mL (56.52 g) of dimethylformamide (DMF) in a 250 mL glass reactor, and the mixture is stirred at room temperature until the solids are dissolved. Diisopropylethylamine (DIEA) (6.49 g, 55 mmol) is added slowly to the reactor contents over a period of from about fifteen to thirty minutes. The resulting mixture is allowed to react at room temperature for about two hours. After this period of time, an aliquot sample of the reaction mixture is taken for analysis of the completeness of the reaction. If the analysis indicates that the reaction is incomplete, the reaction mixture is stirred at room temperature for an additional hour and the reaction mixture is analyzed again. When this analysis indicates the coupling reaction is complete, the reactor contents are held at room temperature with slow stirring.

Steps 2a-3a) Scavenging/Sequestration of Excess Reactant Z-Phe-OSu

Aminomethyl resin (8.30 g) is mixed with 50 mL (47.10 g) of DMF in a second reactor vessel. The resulting mixture is stirred at room temperature until a homogeneous slurry is obtained. The resin/DMF slurry is charged to a glass column, the resin slurry is allowed to settle into a packed bed, and any excess DMF is drained from the column.

The DMF reaction mixture containing the Z-Phe-Gly-OMe product and excess Z-Phe-OSu reagent from Step 1a is then circulated repetitively through the resin column for about one hour in order to remove excess Z-Phe-OSu. Circulation is continued, if necessary, until analysis indicates complete removal of the excess reactant. At this point, the DMF reaction solution containing the blocked Z-Phe-Gly-OMe dipeptide product is set aside. The resin column is then washed by recirculating 20 mL (15.66 g) of *iso*-propanol through the column for about thirty minutes. The *iso*-propanol solution, containing blocked dipeptide product washed from the column, is set aside. The column is next washed by recirculating 40 mL (37.68 g) of DMF through the column for about thirty minutes. The DMF solution, containing blocked dipeptide product washed from the column, is set aside. The resin column is given a final wash by recirculating 20 mL (15.66 g) of *iso*-propanol through the column for about thirty minutes. The DMF reaction solution of blocked dipeptide, the DMF wash solution, and the two *iso*-propanol wash solutions are combined.

Step 4a) N-Terminal Deprotection of the Benzyloxycarbonyl-Phenylalanyl-Glycine-methyl Ester Product of Step 1a

Palladium-Deloxan® (0.90 g) and *para*-toluene sulfonic acid (pTSA) (10 g, 52.05 mmol) are placed in a 1-liter hydrogenator vessel. The hydrogenator vessel is flooded with argon, and the combined DMF/*iso*-propanol solutions from Step 2a-3a are charged to the hydrogenator vessel. The vessel is sealed and evacuated to a pressure of 20-25 inches of mercury (67.7 - 84.6 kPa) and purged three times with hydrogen. Hydrogen is then charged to the hydrogenator vessel to a pressure of 35-45 psi (234.5-310.3 kPa) and the mixture is stirred at about 30°C for about 2 hours.

The hydrogen is then vented from the hydrogenator vessel, and the vessel is purged twice with nitrogen. An aliquot sample of the reaction mixture is taken for analysis of the completeness of reaction. If the analysis indicates incomplete reaction, the hydrogenator vessel is purged twice with hydrogen, recharged with hydrogen to a pressure of 35-45 psi (234.5-310.3 kPa), and the mixture is again stirred at a temperature of about 30°C for an additional one hour. The hydrogenator vessel is evacuated and then purged twice with nitrogen, and an aliquot sample of the reaction mixture is again taken for analysis of the completeness of reaction. The above steps of hydrogenation and reaction mixture analysis are repeated until the analysis indicates substantial completion of the hydrogenolysis reaction.

Upon completion of the hydrogenolysis reaction, the hydrogen is vented from the hydrogenator vessel, the vessel is purged with nitrogen, and the vessel contents are filtered to remove the catalyst. The hydrogenator vessel is rinsed with DMF, and the rinse solution is added to the reaction mixture filtrate.

Step 1b) Preparation of Benzyloxycarbonyl-Lysyl(*tert*-butyloxycarbonyl)-Phenylalanyl-Glycine-methyl Ester

Z-Lys(Boc)-OSu (26.20 g, 55 mmol), DIEA (6.49 g, 55 mmol) and the DMF/*iso*-propanol solution of the N-terminal deprotected dipeptide product of Step 4a (50 mmol, assuming complete reaction) are placed in a 1-liter glass reactor, and the mixture is stirred at room temperature until all solids are dissolved. The mixture is then stirred and allowed to react at room temperature for about two hours. At the end of this time, an aliquot sample of the reaction mixture is taken for analysis of the completeness of reaction. If the analysis indicates that the reaction is incomplete, the mixture is stirred at room temperature for an additional hour and the reaction mixture is again analyzed. When this analysis indicates the coupling reaction is complete, the reactor contents are held at room temperature with slow stirring.

Steps 2b-3b) Scavenging/Sequestration of Excess Reactant Z-Lys(Boc)-OSu

A column of aminomethyl resin (8.30 g) is prepared as described above in step 2a-3a, and the DMF/*iso*-propanol reaction mixture from Step 1b is circulated repetitively through the resin column for about one hour, periodically analyzing the column eluate for absence of excess Z-Lys(Boc)-OSu. When the analysis indicates substantially pure product, the DMF/*iso*-propanol reaction solution of the blocked tripeptide product is set aside, and the column is washed by recirculating 20 mL (15.66 g) of *iso*-propanol through the column for about thirty minutes. The *iso*-propanol solution, containing blocked tripeptide product washed from the column, is set aside. The column is next washed by recirculating 40 mL (37.68 g) of DMF through the column for about thirty minutes. The DMF solution, containing blocked tripeptide product washed from the column, is set aside. The resin column is given a final wash by recirculating 20 mL (15.66 g) of *iso*-propanol through the column for about thirty minutes. The DMF/*iso*-propanol reaction solution of blocked tripeptide product and the *iso*-propanol and DMF wash solutions are combined.

Step 4b N-Terminal Deprotection of the Benzyloxycarbonyl-Lysyl(*tert*-butyloxycarbonyl)-Phenylalanyl-Glycine-methyl Ester Product of Step 1b

Using the same process as that of Step 4a, but without the addition of pTSA, the benzyloxycarbonyl protecting group on the N-terminus of the Z-Lys(Boc)-Phe-Gly-OMe tripeptide product of Step 1b is removed by hydrogenolysis.

Step 1c) Preparation of Benzyloxycarbonyl-Lysyl(*tert*-butyloxycarbonyl)-Lysyl(*tert*-butyloxycarbonyl)-Phenylalanyl-Glycine-methyl Ester

Z-Lys(Boc)-OSu (26.20 g, 55 mmol) and the DMF/*iso*-propanol solution of the deprotected tripeptide product of Step 4b are placed in a 1-liter glass reactor, and the mixture is stirred at room temperature until all solids are dissolved. The mixture is then stirred and allowed to react at room temperature for about two hours. At the end of this time, an aliquot sample of the reaction mixture is taken for analysis of the completeness of reaction. If the analysis indicates that the reaction is incomplete, the mixture is stirred at room temperature for an additional hour and the reaction mixture is again analyzed. When this analysis indicates the coupling reaction is complete, the reactor contents are held at room temperature with slow stirring.

Steps 2c-3c) Scavenging/Sequestration of Excess Reactant Z-Lys(Boc)-OSu

A column of aminomethyl resin (8.30 g) is prepared as described above in Step 2a-3a, and the DMF/*iso*-propanol reaction mixture from Step 1c is circulated repetitively through the resin column for about one hour, periodically analyzing the column eluate for absence of excess Z-Lys(Boc)-OSu. When the analysis indicates substantially pure product, the DMF/*iso*-propanol solution of reaction product is set aside, and the column is washed by recirculating 20 mL (15.66 g) of *iso*-propanol through the column for about thirty minutes. The *iso*-propanol solution, containing blocked tetrapeptide product washed from the column, is set aside. The column is next washed by recirculating 40 mL (37.68 g) of DMF through the column for about thirty minutes. The DMF solution, containing blocked tetrapeptide product washed from the column, is set aside. The resin column is given a final wash by recirculating 20 mL (15.66 g) of *iso*-propanol through the column for about thirty minutes. The DMF/*iso*-propanol reaction solution of blocked tetrapeptide product and the *iso*-propanol and DMF wash solutions are combined.

Step 4c) N-Terminal Deprotection of the Benzyloxycarbonyl-Lysyl(*tert*-butyloxycarbonyl)-Lysyl(*tert*-butyloxycarbonyl)-Phenylalanyl-Glycine-methyl Ester Product of Step 1c

Using the same process as that of Step 4a, but without the addition of pTSA, the benzyloxycarbonyl protecting group on the N-terminus of the Z-Lys(Boc)-Lys(Boc)-Phe-Gly-OMe tetrapeptide product of Step 1c is removed by hydrogenolysis.

Step 1d) Preparation of Benzyloxycarbonyl-Alanyl-Lysyl(*tert*-butyloxycarbonyl)-Lysyl(*tert*-butyloxycarbonyl)-Phenylalanyl-Glycine-methyl Ester

Z-Ala-OSu (16.00 g, 50 mmol) and the DMF/*iso*-propanol solution of the deprotected tetrapeptide product of Step 4c are placed in a 1-liter glass reactor, and the mixture is stirred at room temperature until all solids are dissolved. The mixture is then stirred and allowed to react at room temperature for about two hours. At the end of this time, an aliquot sample of the reaction mixture is taken for analysis of the completeness of reaction. If the analysis indicates that the reaction is incomplete, the mixture is stirred at room temperature for an additional hour and the reaction mixture is again analyzed. When this analysis indicates the coupling reaction is complete, the reactor contents are held at room temperature with slow stirring. (Note: Because a theoretical excess of Z-Ala-OSu was not used in this particular coupling reaction, Step 2d was not required, and was not conducted.)

Step 3d) Precipitation/Crystallization of Condensation Product

To the DMF/*iso*-propanol (± 500 mL) solution of the blocked pentapeptide from Step 1d is added precooled water (250 mL), causing precipitation/crystallization of the Z-Ala-Lys(Boc)-Lys(Boc)-Phe-Gly-OMe pentapeptide product. The pentapeptide is isolated by filtration, rinsed with DMF/water (500/250 mL), then with 2-liters of water. The resulting solid is dissolved again in DMF (700 mL), precipitated/crystallized by addition of water (350 mL), collected by filtration and dried under vacuum. The yield of Z-Ala-Lys(Boc)-Lys(Boc)-Phe-Gly-OMe is 28.26 g (62% of theoretical yield).

Step 4d) N-Terminal Deprotection of the Benzyloxycarbonyl-Alanyl-Lysyl(*tert*-butyloxycarbonyl)-Lysyl(*tert*-butyloxycarbonyl)-Phenylalanyl-Glycine-methyl Ester Product of Step 3d

Z-Ala-Lys(Boc)-Lys(Boc)-Phe-Gly-OMe (16.90 g, 18.84 mmol) is mixed with 160 mL of NMP in a 1-liter glass reactor. Then, using the same process as that of Step 4a, but without the addition of pTSA, the benzyloxycarbonyl protecting group on the N-terminus of the blocked pentapeptide product (of Step 3d) is removed by hydrogenolysis.

Step 1e) Preparation of Benzyloxycarbonyl-Lysyl(*tert*-butyloxycarbonyl)-Alanyl-Lysyl(*tert*-butyloxycarbonyl)-Lysyl(*tert*-butyloxycarbonyl)-Phenylalanyl-Glycine-methyl Ester

Z-Lys(Boc)-OSu (9.89 g, 20.72 mmol) and DIEA (1.22 g, 10.36 mmol) are mixed in a 1-liter glass reactor vessel with the NMP solution of the N-terminal deprotected pentapeptide

product from Step 4d. The mixture is stirred at room temperature until all solids are dissolved. The resulting mixture is allowed to react at room temperature for at least 2 hours, at which time an aliquot sample of the reaction mixture is analyzed for completeness of reaction. If the analysis indicates that the reaction is incomplete, the mixture is stirred at room temperature for an additional hour and the reaction mixture is again analyzed. When this analysis indicates the coupling reaction is complete, the reactor contents are held at room temperature with slow stirring.

Steps 2e-3e) Scavenging/Sequestration of Excess Reactant Z-Lys(Boc)-OSu

A column of aminomethyl resin (3.45 g) is prepared as described above in Step 2a-3a, and the NMP reaction mixture of the blocked hexapeptide from Step 1e is then circulated repetitively through the resin column for about one hour in order to remove excess Z-Lys(Boc)-OSu. Circulation is continued, if necessary, until analysis indicates complete removal of the excess reactant. At this point, the NMP reaction solution containing the blocked hexapeptide product is set aside. The resin column is then washed by recirculating 20 mL (15.66 g) of *iso*-propanol through the column for about thirty minutes. The *iso*-propanol solution, containing blocked hexapeptide product washed from the column, is set aside. The column is next washed by recirculating 40 mL (41.32 g) of NMP through the column for about thirty minutes. The NMP solution, containing blocked hexapeptide product washed from the column, is set aside. The resin column is given a final wash by recirculating 20 mL (15.66 g) of *iso*-propanol through the column for about thirty minutes. The NMP reaction solution of blocked hexapeptide, the NMP wash solution, and the two *iso*-propanol wash solutions are combined.

Step 4e) N-Terminal Deprotection of the Benzyloxycarbonyl-Lysyl(*tert*-butyloxycarbonyl)-Alanyl-Lysyl(*tert*-butyloxycarbonyl)-Lysyl(*tert*-butyloxycarbonyl)-Phenylalanyl-Glycine-methyl Ester Product of Step 1e

Using the same process as that of Step 4a, but without the addition of pTSA, the benzyloxycarbonyl protecting group on the N-terminus of the Z-Lys(Boc)-Ala-Lys(Boc)-Lys(Boc)-Phe-Gly-OMe hexapeptide product of Step 1e is removed by hydrogenolysis.

Step 1f) Preparation of Benzyloxycarbonyl-Lysyl(*tert*-butyloxycarbonyl)-Lysyl(*tert*-butyloxycarbonyl)-Alanyl-Lysyl(*tert*-butyloxycarbonyl)-Lysyl(*tert*-butyloxycarbonyl)-Phenylalanyl-Glycine-methyl Ester

Z-Lys(Boc)-OSu (9.89 g, 20.72 mmol) and the NMP/*iso*-propanol solution of the deprotected hexapeptide product of Step 4e are placed in a 1-liter glass reactor, and the mixture is stirred at room temperature until all solids are dissolved. The resulting mixture allowed to react at

room temperature for about two hours, at which time an aliquot sample of the reaction mixture is taken for analysis of the completeness of reaction. If the analysis indicates that the reaction is incomplete, the mixture is stirred at room temperature for an additional hour and the reaction mixture is again analyzed. When this analysis indicates the coupling reaction is complete, the reactor contents are held at room temperature with slow stirring.

Steps 2f-3f) Scavenging/Sequestration of Excess Reactant Z-Lys(Boc)-OSu

A column of aminomethyl resin (3.45 g) is prepared as described above in Step 2a-3a, and the NMP/*iso*-propanol reaction mixture from Step 1f is circulated repetitively through the resin column for about one hour, periodically analyzing the column eluate for absence of excess Z-Lys(Boc)-OSu. When the analysis indicates substantially pure product, the NMP/*iso*-propanol solution of reaction product is set aside, and the column is washed by recirculating 40 mL (31.32 g) of *iso*-propanol through the column for about thirty minutes. The *iso*-propanol solution, containing blocked heptapeptide product washed from the column, is set aside. The column is next washed by recirculating 80 mL (82.64 g) of NMP through the column for about thirty minutes. The NMP solution, containing blocked heptapeptide product washed from the column, is set aside. The resin column is given a final wash by recirculating 40 mL (31.32 g) of *iso*-propanol through the column for about thirty minutes. The NMP/*iso*-propanol reaction solution of blocked heptapeptide product, and the *iso*-propanol and NMP wash solutions are combined.

Step 4f) N-Terminal Deprotection of the Benzyloxycarbonyl-Lysyl(*tert*-butyloxycarbonyl)-Lysyl(*tert*-butyloxycarbonyl)-Alanyl-Lysyl(*tert*-butyloxycarbonyl)-Lysyl(*tert*-butyloxycarbonyl)-Phenylalanyl-Glycine-methyl Ester Product of Step 1f

Using the same process as that of Step 4a, but without the addition of pTSA, the benzyloxycarbonyl protecting group on the N-terminus of the Z-Lys(Boc)-Lys(Boc)-Ala-Lys(Boc)-Lys(Boc)-Phe-Gly-OMe heptapeptide product of Step 1f is removed by hydrogenolysis.

Step 1g) Preparation of Benzyloxycarbonyl-Leucyl-Lysyl(*tert*-butyloxycarbonyl)-Lysyl(*tert*-butyloxycarbonyl)-Alanyl-Lysyl(*tert*-butyloxycarbonyl)-Lysyl(*tert*-butyloxycarbonyl)-Phenylalanyl-Glycine-methyl Ester

Z-Leu-OSu (7.50 g, 20.72 mmol) and the NMP/*iso*-propanol solution of the deprotected heptapeptide product of Step 4f are placed in a 1-liter glass reactor, and the mixture is stirred at room temperature until all solids are dissolved. The mixture is then stirred and allowed to react at room temperature for about two hours. At the end of this time, an aliquot sample of the reaction mixture is taken for analysis of the completeness of reaction. If the analysis indicates that the

reaction is incomplete, the mixture is stirred at room temperature for an additional hour and the reaction mixture is again analyzed. When this analysis indicates the coupling reaction is complete, the reactor contents are held at room temperature with slow stirring.

Steps 2g-3g) Scavenging/Sequestration of Excess Reactant Z-Leu-OSu and Precipitation/Crystallization of the Condensation Product

A column of aminomethyl resin (3.45 g) is prepared as described above in Step 2a-3a, and the NMP/*iso*-propanol reaction mixture from Step 1g is circulated repetitively through the resin column for about one hour, periodically analyzing the column eluate for absence of excess Z-Leu-OSu. When the analysis indicates substantially pure product, the NMP/*iso*-propanol solution of reaction product is set aside, and the column is washed by recirculating 40 mL (31.32 g) of *iso*-propanol through the column for about thirty minutes. The *iso*-propanol solution, containing blocked octapeptide product washed from the column, is set aside. The column is next washed by recirculating 80 mL (81.64 g) of NMP through the column for about thirty minutes. The NMP solution, containing blocked octapeptide product washed from the column, is set aside The resin column is given a final wash by recirculating 40 mL (31.32 g) of *iso*-propanol through the column for about thirty minutes. The NMP/*iso*-propanol reaction solution of blocked octapeptide product, and the *iso*-propanol and NMP wash solutions are combined.

To the NMP/*iso*-propanol (± 500 mL) solution of the blocked octapeptide is added precooled water (200 mL), causing precipitation/crystallization of the Z-Leu-Lys(Boc)-Lys(Boc)-Ala-Lys(Boc)-Lys(Boc)-Phe-Gly-OMe octapeptide product. The octapeptide is isolated by filtration, rinsed with NMP/water (500/200 mL) and 2 liters of water, then dried under vacuum. The yield of Z-Leu-Lys(Boc)-Lys(Boc)-Ala-Lys(Boc)-Lys(Boc)-Phe-Gly-OMe is 22.75 g (82% of theoretical yield from Z-Ala-Lys(Boc)-Lys(Boc)-Phe-Gly-OMe).

Step 4g) N-Terminal Deprotection of the Benzyloxycarbonyl-Leucyl-Lysyl(*tert*-butyloxycarbonyl)-Lysyl(*tert*-butyloxycarbonyl)-Alanyl-Lysyl(*tert*-butyloxycarbonyl)-Lysyl(*tert*-butyloxycarbonyl)-Phenylalanyl-Glycine-methyl Ester Product of Step 3g

Z-Leu-Lys(Boc)-Lys(Boc)-Ala-Lys(Boc)-Lys(Boc)-Phe-Gly-OMe (19.18 g, 13.08 mmol) is mixed with 200 mL of NMP in a 1-liter glass reactor. Then, using the same process as that of Step 4a, but without the addition of pTSA, the benzyloxycarbonyl protecting group on the N-terminus of the blocked octapeptide product (of Step 3g) is removed by hydrogenolysis.

Step 1h) Preparation of Benzyloxycarbonyl-Phenylalanyl-Leucyl-Lysyl(*tert*-butyloxycarbonyl)-Lysyl(*tert*-butyloxycarbonyl)-Alanyl-Lysyl(*tert*-

butyloxycarbonyl)-Lysyl(*tert*-butyloxycarbonyl)-Phenylalanyl-Glycine-methyl Ester

Z-Phe-OSu (5.70 g. 14.38 mmol) is mixed in a 1-liter glass reactor vessel with the NMP solution of the N-terminal deprotected octapeptide product from Step 4g. The mixture is stirred at room temperature until all solids are dissolved. The resulting mixture is allowed to react at room temperature for at least 2 hours, at which time an aliquot sample of the reaction mixture is analyzed for completeness of reaction. If the analysis indicates that the reaction is incomplete, the mixture is stirred at room temperature for an additional hour and the reaction mixture is again analyzed. When this analysis indicates the coupling reaction is complete, the reactor contents are held at room temperature with slow stirring.

Steps 2h-3h) Scavenging/Sequestration of Excess Reactant Z-Phe-OSu

A column of aminomethyl resin (2.40 g) is prepared as described above in Step 2a-3a, and the NMP reaction mixture of the blocked nonapeptide from Step 1h is then circulated repetitively through the resin column for about one hour in order to remove excess Z-Phe-OSu. Circulation is continued, if necessary, until analysis indicates complete removal of the excess reactant. At this point, the NMP reaction solution containing the blocked nonapeptide product is set aside. The resin column is then washed by recirculating 20 mL (15.66 g) of *iso*-propanol through the column for about thirty minutes. The *iso*-propanol solution, containing blocked nonapeptide product washed from the column, is set aside. The column is next washed by recirculating 40 mL (41.32 g) of NMP through the column for about thirty minutes. The NMP solution, containing blocked nonapeptide product washed from the column, is set aside. The resin column is given a final wash by recirculating 20 mL (15.66 g) of *iso*-propanol through the column for about thirty minutes. The NMP reaction solution of blocked nonapeptide, the NMP wash solution, and the two *iso*-propanol wash solutions are combined.

Step 4h) N-Terminal Deprotection of the Benzyloxycarbonyl-Phenylalanyl-Leucyl-Lysyl(*tert*-butyloxycarbonyl)-Lysyl(*tert*-butyloxycarbonyl)- Alanyl-Lysyl(*tert*-butyloxycarbonyl)-Lysyl(*tert*-butyloxycarbonyl)-Phenylalanyl-Glycine-methyl Ester Product of Step 1h

Using the same process as that of Step 4a, but without the addition of pTSA, the benzyloxycarbonyl protecting group on the N-terminus of the Z-Phe-Leu-Lys(Boc)-Lys(Boc)-Ala-Lys(Boc)-Lys(Boc)-Phe-Gly-OMe nonapeptide product of Step 1h is removed by hydrogenolysis.

Step 1i) Preparation of Benzyloxycarbonyl-Lysyl(*tert*-butyloxycarbonyl)-Phenylalanyl-Leucyl-Lysyl(*tert*-butyloxycarbonyl)-Lysyl(*tert*-

butyloxycarbonyl)-Alanyl-Lysyl(*tert*-butyloxycarbonyl)-Lysyl(*tert*-butyloxycarbonyl)-Phenylalanyl-Glycine-methyl Ester

Z-Lys(Boc)-OSu (6.55 g, 13.73 mmol) and the NMP/*iso*-propanol solution of the deprotected nonapeptide product of Step 4h are placed in a 1-liter glass reactor, and the mixture is stirred at room temperature until all solids are dissolved. The resulting mixture is allowed to react at room temperature for about two hours, at which time an aliquot sample of the reaction mixture is taken for analysis of the completeness of reaction. If the analysis indicates that the reaction is incomplete, the mixture is stirred at room temperature for an additional hour and the reaction mixture is again analyzed. When this analysis indicates the coupling reaction is complete, the reactor contents are held at room temperature with slow stirring. (Note: Scavenging/sequestration of excess reactant Z-Lys(Boc)-OSu, as per a final Step 2i-3i sequence, was not conducted prior to isolation of the final condensation product in Example 2.)

Step 5) Isolation of Benzyloxycarbonyl-Lysyl(*tert*-butyloxycarbonyl)-Phenylalanyl-Leucyl-Lysyl(*tert*-butyloxycarbonyl)-Lysyl(*tert*-butyloxycarbonyl)-Alanyl-Lysyl(*tert*-butyloxycarbonyl)-Lysyl(*tert*-butyloxycarbonyl)-Phenylalanyl-Glycine-methyl Ester

To the NMP/*iso*-propanol (± 260 mL) solution of the blocked decapeptide from Step 1i is added precooled water (150 mL), causing precipitation/crystallization of the Z-Lys(Boc)-Phe-Leu-Lys(Boc)-Lys(Boc)-Ala-Lys(Boc)-Lys(Boc)-Phe-Gly-OMe decapeptide product. The decapeptide is isolated by filtration, rinsed with 1 liter of methanol, and dried under vacuum. The yield of Z-Lys(Boc)-Phe-Leu-Lys(Boc)-Lys(Boc)-Ala-Lys(Boc)-Lys(Boc)-Phe-Gly-OMe is 19.02 g (79% of theoretical yield from Z-Leu-Lys(Boc)-Lys(Boc)-Ala-Lys(Boc)-Lys(Boc)-Phe-Gly-OMe, 41% overall yield).

Example 2

Preparation of 9-Fluorenylmethoxycarbonyl-*beta*-Alanyl-Leucyl-Alanyl-Leucine-*tert*-butyl Ester

Step 1) Preparation of Benzyloxycarbonyl-Alanyl-Leucine- *tert*-butyl Ester

Leu-O*t*Bu·HCl (184 g, 0.82 mol), and Z-Ala-OSu (290 g, 0.91 mol) are mixed with 1.9 liters (1790 g) of dimethylformamide in a 5-liter glass reactor, and the mixture is stirred at room temperature until the solids are dissolved. Diisopropylethylamine (115 g (0.97 mol) is added slowly to the reactor contents over a period of from about fifteen to thirty minutes. The resulting mixture is allowed to react at room temperature for about two hours. Aliquot samples of the reactor contents are then taken for analysis of the completeness of the coupling reaction. When

these analyses indicate the reaction is complete, the reactor contents are held at room temperature with slow stirring.

Steps 2-3) Scavenging/Sequestration of Excess Reactant (Z-Ala-Osu)

Aminomethyl resin (164 g) is mixed with 1.6 liters (1550 g) of dimethylformamide in a second reactor vessel. The resulting mixture is stirred at room temperature until a homogeneous slurry is obtained. The resin/DMF slurry is charged to a glass column, the resin slurry is allowed to settle into a packed bed, and any excess DMF is drained from the column.

The reaction mixture containing the Z-Ala-Leu-OtBu dipeptide product and excess Z-Ala-OSu reagent is then circulated repetitively through the resin column for about one hour. At the end of this time, the DMF solution containing the Z-Ala-Leu-O-*t*-Bu dipeptide product is set aside, and an aliquot sample is removed for verification of the completeness of the scavenging reaction. Circulation is continued, if necessary, until analysis indicates complete removal of the excess reactant. The resin column is then washed by recirculating 785 mL (615 g) of *iso*-propanol through the column for about thirty minutes. The *iso*-propanol solution, containing the Z-Ala-Leu-O-*t*-Bu dipeptide product washed from the column is set aside. The column is next washed by recirculating 1.56 liters (1470 g) of dimethyl-formamide through the column for about thirty minutes. The DMF solution, containing the Z-Ala-Leu-O-*t*-Bu dipeptide product washed from the column is set aside. The resin column is given a final wash by recirculating 785 mL (615 g) of *iso*-propanol through the column for about thirty minutes. The original reaction solution in DMF, the DMF wash solution, and the two *iso*-propanol wash solutions are combined.

Step 4) - N-Terminal Deprotection of the Benzyloxycarbonyl-Alanyl-Leucine-tert-butyl Ester Product of Step 1)

Palladium-Deloxan® (16 g) is placed in a 2-gallon (7.57 L) hydrogenator vessel. The hydrogenator vessel is flooded with argon, and the combined DMF/*iso*-propanol solutions from steps (2) and (3) are charged to the hydrogenator. The vessel is sealed and evacuated to a pressure of 20-25 inches of mercury (67.7 - 84.6 kPa) and purged three times with hydrogen. Hydrogen is then charged to the hydrogenator vessel to a pressure of 35-45 psi (234.5-310.3 kPa) and the mixture is stirred at about 30°C for about 2 hours.

The hydrogenator vessel is then vented to vacuum, repressurized with hydrogen to a pressure of 35-45 psi (234.5-310.3 kPa), and hydrogenation is continued for about one hour at 30°C . The vessel is the purged and evacuated twice with nitrogen, and an aliquot sample of the reaction mixture is taken for analysis of the completeness of reaction. If the analysis indicates

incomplete reaction, the hydrogenator vessel is purged twice with hydrogen, recharged with hydrogen to a pressure of 35-45 psi (234.5-310.3 kPa) and the mixture is again stirred at a temperature of about 30°C for an additional hour. The hydrogenator vessel is evacuated and then purged twice with nitrogen, and an aliquot sample of the reaction mixture is again taken for analysis of the completeness of reaction. The above steps of hydrogenation and reaction mixture analysis are repeated until the analysis indicates substantial completion of the hydrogenolysis reaction.

Upon completion of the hydrogenolysis reaction, the hydrogenator vessel is vented to vacuum, purged with nitrogen, and the vessel contents are filtered to remove the catalyst. The hydrogenator vessel is rinsed with dimethylformamide, and the rinse solution is added to the reaction mixture filtrate.

Step 1') - Preparation of Benzyloxycarbonyl-Leucyl-Alanyl-Leucine- *tert*-butyl Ester

Z-Leu-OSu (328 g, 0.90 mol) and the solution of the deprotected dipeptide product of step 4) (0.82 mol, assuming complete reaction) are placed in a 12-liter glass reactor, and the mixture is stirred at room temperature until all solids are dissolved. The mixture is then stirred at room temperature for about two hours. At the end of this time, an aliquot sample of the reaction mixture is taken for analysis of the completeness of the coupling reaction. If the analysis indicates that the reaction is incomplete, the mixture is stirred at room temperature for an additional hour and the reaction mixture is again analyzed. When these analyses indicate the coupling reaction is complete, the reactor contents are held at room temperature with slow stirring.

Steps 2'-3') - Scavenging/Sequestration of Excess Reactant Z-Leu-OSu

A column of aminomethyl resin (164 g) is prepared as described above in steps 2) and 3) and the reaction mixture from step 1') is circulated repetitively through the resin column for about one hour, periodically analyzing the column eluate for absence of excess Z-Leu-OSu. When the analysis indicates substantially pure tripeptide product, the solution of Z-Leu-Ala-Leu-O-*t*-Bu is set aside, and the column is washed by recirculating 785 mL (615 g) of *iso*-propanol through the column for about thirty minutes. The *iso*-propanol solution, containing Z-Leu-Ala-Leu-O-*t*-Bu tripeptide product washed from the column, is set aside. The column is next washed by recirculating 1.56 liters (1470 g) of dimethylformamide through the column for about thirty minutes. The DMF solution, containing of Z-Leu-Ala-Leu-O-*t*-Bu tripeptide product washed from the column is set aside. The resin column is given a final wash by circulating 785 mL (615 g) of *iso*-propanol through the column for about thirty minutes. The original solution of of Z-Leu-Ala-

Leu-O-*t*-Bu tripeptide product, the two *iso*-propanol wash solutions, and the DMF wash solution are combined.

Step 4') - N-Terminal Deprotection of the Benzyloxycarbonyl-Leucyl-Alanyl-Leucine - *tert*-butyl Ester Product of Step 1')

Using the same process as that in of step 4), the benzyloxycarbonyl protecting group on the N-terminus of the Z-Leu-Ala-Leu-O-*t*-Bu tripeptide product of step 1') is removed by hydrogenolysis.

Step 1") - Preparation of 9-Fluorenylmethoxycarbonyl-*beta*-Alanyl-Leucyl-Alanyl-Leucine-*tert*-Butyl Ester

Fmoc-*beta*-alanine-N-hydroxysuccinimide ester (Fmoc-β-Ala-OSu, 336 g, 0.82 mol) is mixed in a 50-liter glass reactor vessel with the DMF/*iso*-propanol solution of the N-terminal deprotected tripeptide product from step 4'). The mixture is stirred at room temperature until all solids are dissolved. The resulting mixture is stirred at room temperature for at least 2 hours, at which time an aliquot sample of the reaction mixture is analyzed for completeness of the coupling reaction. If the analysis indicates that the reaction is incomplete, the mixture is stirred at room temperature for an additional hour after which the reaction mixture is again analyzed.

When these analyses indicate the reaction is complete, pre-cooled distilled water is added to the 50-liter reactor vessel, maintaining the temperature below 25°C, causing the precipitation/crystallization of the Fmoc-β-Ala-Leu-Ala-Leu-O-*t*-Bu tetrapeptide product. The quantity of distilled water added is calculated using the equation:

Kg of Distilled Water = [(Volume of Solution)/0.55] – [Volume of Solution].

The resulting slurry of tetrapeptide is mixed well in the 50-liter vessel at room temperature, after which time dimethylformamide/water (55:45, v/v) is added to aid in the flowability of the slurry. Mixing is continued for about two hours at room temperature, after which time the tetrapeptide product is isolated by filtration with collection of the mother liquor in an appropriate receiver. An additional 4 kg of dimethylformamide/water (55:45/ v/v) is added to the 50-liter reactor vessel to rinse out residual tetrapeptide product, which is isolated by filtration, with collection of the filtrate in an appropriate receiver. Approximately 4 kg of distilled water is then added to the 50-liter glass reactor to rinse out the remaining tetrapeptide product which is likewise isolated by filtration with collection of the filtrate in an appropriate receiver. Sample of the distilled water filtrate is analyzed for the presence of dimethylformamide; distilled water is used to wash the combined tetrapeptide filter cake until no dimethylformamide is detected in the filtrate.

At this point, the filter cake is washed with approximately 4 kg of acetonitrile, with the filtrate wash being collected in an appropriate receiver. The acetonitrile-wet filter cake is aspirated to dryness for about one hour, and then transferred to a glass drying tray and dried under vacuum and nitrogen flow at 30°C. The yield of Fmoc-β-Ala-Leu-Ala-Leu-O-*t*-Bu is 30.5 g (60% of theoretical yield).

WE CLAIM:

1. A process for the synthesis of a polypeptide having a pre-determined number and sequence of amino acid residues comprising, sequentially, the steps of:

a) exposing, in solution, a first substrate amino acid or peptide fragment of said polypeptide, said first substrate amino acid or peptide fragment being protected at either of its N- or C-terminus, to a stoichiometric excess of a second reactant amino acid or peptide fragments of said polypeptide, said second reactant amino acid or peptide fragment being protected at the other of its N- or C-terminus, to form a condensation product of said substrate and reactant, said condensation product being protected at both its N- and C-termini;

b) contacting the solution from step a) with an insoluble scavenger having a reactive functionality complementary to the unprotected N- or C-terminal functionality of said first substrate amino acid or peptide fragment, to sequester the excess of said second reactant amino acid or peptide fragment;

c) removing the sequestered excess second reactant amino acid or peptide fragment, leaving the condensation product and reaction by-products in solution; or conducting subsequent precipitation or crystallization of the condensation product, then redissolving the condensation product if synthesis continuation is necessary or desired;

d) removing the protecting group from either the N- or C-terminus of the condensation product of step a);

e) repeating steps a) through d) if the desired polypeptide sequence is not yet achieved, with the deprotected condensation product of each previous step d) becoming the substrate peptide fragment of each successive step a); and

f) isolating and deprotecting, if needed, the final polypeptide product once the desired peptide sequence is achieved.

2. The process according to Claim 1 wherein the protecting group removed in step d) is removed from the N-terminus.

3. The process according to Claim 1 wherein the protecting group removed in step d) is removed from the C-terminus.

4. The process according to Claim 1 wherein the insoluble scavenger employed in step b) is selected from amine- and carboxyl-functionalized resins.

5. The process according to Claim 2 wherein the insoluble scavenger employed in step b) is an amine-functionalized resin.

6. The process according to Claim 3 wherein the insoluble scavenger employed in step b) is a carboxyl-functionalized resin.

7. The process according to Claim 5 wherein said resin is an aminomethyl-functionalized polystyrene-divinylbenzene resin.

8. A process for the synthesis of a polypeptide having a pre-determined number and sequence of amino acid residues comprising the steps of:

a) exposing a solution of a substrate amino acid or fragment of said polypeptide, said substrate having a C-terminal protecting group not removable by hydrogenolysis, to a stoichiometric excess of a reactant amino acid or a fragment of said polypeptide, said reactant having an N-terminal protecting group removable by hydrogenolysis, to form a condensation product;

b) contacting the solution from step a) with an amine-functionalized resin to sequester the excess of said reactant amino acid or peptide fragment;

c) removing from the solution the sequestered excess of said reactant amino acid or peptide fragment leaving the condensation product and reaction

by-products in solution; or conducting subsequent precipitation or crystallization of the condensation product, then redissolving the condensation product if sysnthesis continuation is necessary or deisired;

d) subjecting the solution from step c) to hydrogenolysis conditions to remove the protecting group from the N-terminus of said condensation product;

e) repeating steps a) through d) utilizing as the substrate peptide fragment for subsequent steps a) the deprotected condensation product of subsequent steps d) until the desired polypeptide has been produced.

9. The process according to Claim 8 wherein said amine-functionalized resin is an aminomethyl-modified resin.

10. The process according to Claim 8 wherein the C-terminus of the reactant amino acid or a peptide fragment is activated for reaction with amino functional groups.

11. The process according to Claim 10 wherein the C-terminus of the reactant amino acid or a peptide fragment is activated by esterification with N-hydroxysuccinimide.

12. The process according to Claim 8 wherein the N-terminal protecting group of the reactant amino acid or peptide fragment is benzyloxycarbonyl.

13. A process for the synthesis of a polypeptide having a pre-determined number and sequence of amino acid residues comprising the steps of:

a) exposing a solution of a substrate amino acid or fragment of said polypeptide, said substrate having a C-terminal protecting group, to a stoichiometric excess of a reactant amino acid or a fragment of said polypeptide, said reactant having an N-terminal benzyloxycarbonyl protecting group and being activated by esterification with N-hydroxy-succinimide, to form a condensation product until reaction between said substrate and reactant is substantially complete;

b) contacting the solution from step a) with an amine-functionalized resin to sequester and to remove from solution the excess of the reactant amino acid or peptide fragment leaving the condensation product and reaction by-products in solution or conducting subsequent precipitation or crystallization of the condensation product. then redissolving the condensation product if synthesis continuation is necessary or desired;

c) subjecting the solution from step b) to catalytic hydrogenation conditions to remove the benzyloxycarbonyl protecting group from the N-terminus of said condensation product;

d) removing, by filtration, the hydrogenation catalyst from the solution;

e) repeating steps a) through d) until the desired polypeptide has been produced; and

f) isolating and deprotecting, if needed or desired, the product polypeptide.

14. A process according to claim 13 wherein the polypeptide synthesized is Benzyloxycarbonyl-Lysyl(*tert*-butyloxycarbonyl)-Alanyl-Phenylalanyl-Valyl-Lysyl(*tert*-butyloxycarbonyl)-Isoleucyl-Leucyl-Lysyl(*tert*-butyloxycarbonyl)-Lysine(*tert*-butyloxycarbonyl)-methyl Ester.

15. A process according to claim 13 wherein the polypeptide synthesized is 9-Fluorenylmethoxycarbonyl-*beta*-Alanyl-Leucyl-Alanyl-Leucine-*tert*-butyl Ester.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/14152

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K1/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, CHEM ABS Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	R J BOOTH & J C HODGES: "Polymer-supported quenching reagents for parallel purification" JOURNAL OF THE AMERICAN CHEMICAL SOCIETY., vol. 119, no. 21, 28 May 1997 (1997-05-28), pages 4882-4886, XP002149792 AMERICAN CHEMICAL SOCIETY, WASHINGTON, DC., US ISSN: 0002-7863 cited in the application the whole document --- -/--	1-15

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 October 2000	27/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Masturzo, P

2

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/14152

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	S D BROWN & R W ARMSTRONG : "Synthesis of tetrasubstituted ethylenes on solid support via resin capture " JOURNAL OF THE AMERICAN CHEMICAL SOCIETY., vol. 118, no. 26, 3 July 1996 (1996-07-03), pages 6331-6332, XP002149793 AMERICAN CHEMICAL SOCIETY, WASHINGTON, DC., US ISSN: 0002-7863 cited in the application the whole document	1-15
A	L M GAYO & M J SUTO: "Ion-exchange resins for solution phase parallel synthesis of chemical libraries" TETRAHEDRON LETTERS., vol. 38, no. 4, 27 January 1997 (1997-01-27), pages 513-516, XP002149794 ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM., NL ISSN: 0040-4020 cited in the application the whole document	1-15
A	WO 97 42230 A (WARNER-LAMBERT) 13 November 1997 (1997-11-13) the whole document	1-15
A	D L FLYNN ET AL.: "Chemical library purification strategy based on principles of complementary molecular reactivity and molecular recognition " JOURNAL OF THE AMERICAN CHEMICAL SOCIETY., vol. 119, no. 21, 28 May 1997 (1997-05-28), pages 4874-4881, XP002149798 AMERICAN CHEMICAL SOCIETY, WASHINGTON, DC., US ISSN: 0002-7863 cited in the application the whole document	1-15

INTERNATIONAL SEARCH REPORT

...rormation on patent family members

International Application No
PCT/US 00/14152

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9742230 A	13-11-1997	AU 2746297 A	26-11-1997
		CA 2251700 A	13-11-1997
		EP 0896590 A	17-02-1999

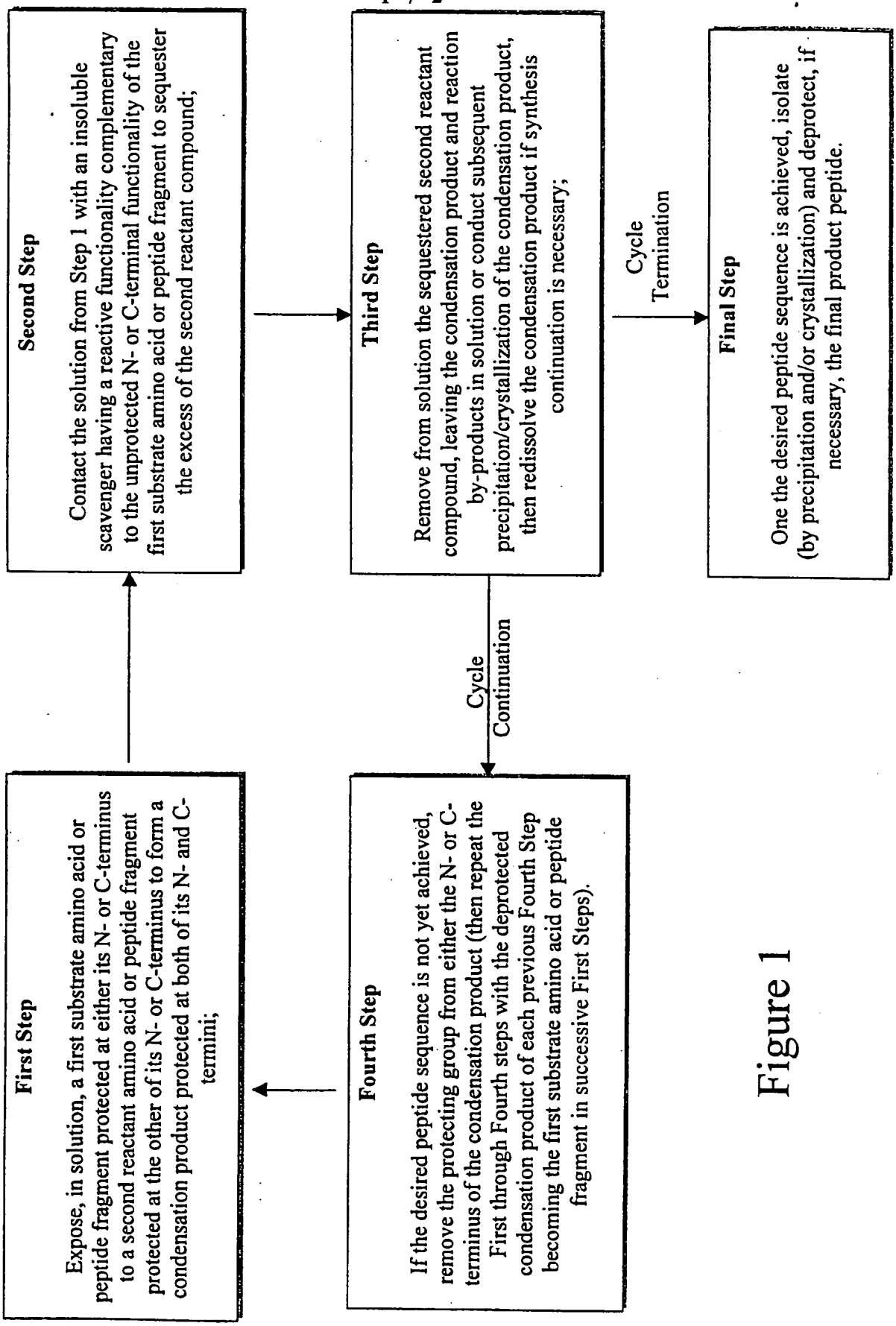

First Step
Expose, in solution, a first substrate amino acid or peptide fragment protected at either its N- or C-terminus to a second reactant amino acid or peptide fragment protected at the other of its N- or C-terminus to form a condensation product protected at both of its N- and C-termini;
Second Step
Contact the solution from Step 1 with an insoluble scavenger having a reactive functionality complementary to the unprotected N- or C-terminal functionality of the first substrate amino acid or peptide fragment to sequester the excess of the second reactant compound;
Third Step
Remove from solution the sequestered second reactant compound, leaving the condensation product and reaction by-products in solution or conduct subsequent precipitation/crystallization of the condensation product, then redissolve the condensation product if synthesis continuation is necessary;
Fourth Step
If the desired peptide sequence is not yet achieved, remove the protecting group from either the N- or C-terminus of the condensation product (then repeat the First through Fourth steps with the deprotected condensation product of each previous Fourth Step becoming the first substrate amino acid or peptide fragment in successive First Steps).
Cycle Continuation
Cycle Termination
Final Step
One the desired peptide sequence is achieved, isolate (by precipitation and/or crystallization) and deprotect, if necessary, the final product peptide.

Figure 1

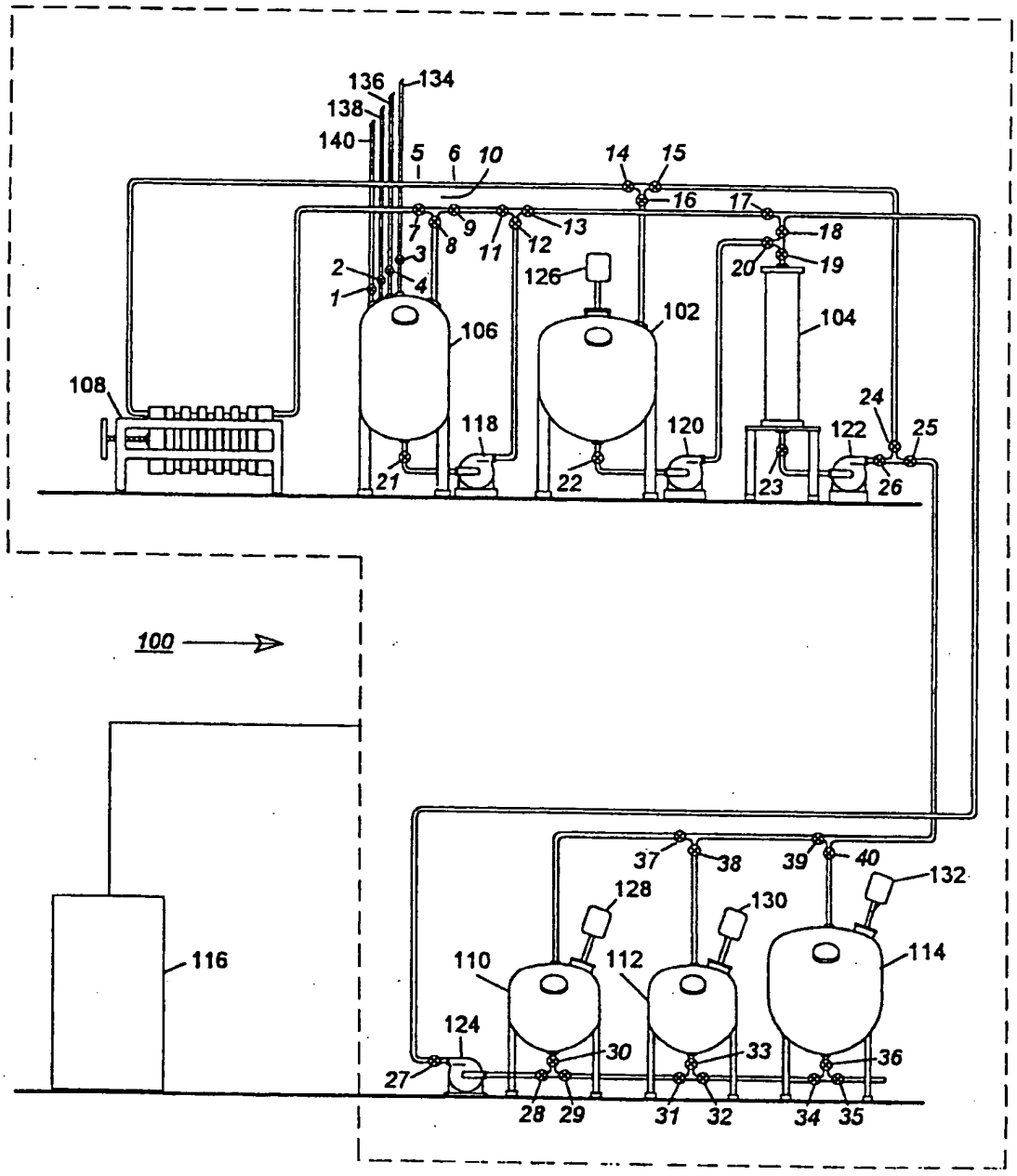

136
134
138
140
5
6
10
14
15
16
17
18
19
20
7
9
8
11
12
13
3
2
4
1
126
102
104
106
108
118
120
122
24
25
21
22
23
26
100
37
38
39
40
132
128
130
110
112
114
116
124
30
33
36
27
28
29
31
32
34
35

Figure 2